Yield and botanical composition of subterranean clover in response to ALS inhibiting herbicides and waterlogging

A thesis

submitted in partial fulfillment

of the requirement for the Degree of

Master of Agricultural Science

at Lincoln University

by

B. J. O. Taylor

Lincoln University

2019

Abstract of a Thesis submitted in partial fulfillment of the requirement for the Degree of Master of Agricultural Science

Yield and botanical composition of subterranean clover in response to ALS inhibiting herbicides and waterlogging

By

B. J. O. Taylor

Subterranean clover is an important component of dryland pastures as it has high growth in early spring and improves soil quality through nitrogen fixation. An issue when establishing subterranean clover swards is broadleaf weed control. This thesis investigated the herbicide tolerance of seven subterranean clover cultivars to two acetolactate synthase (ALS) inhibiting herbicides, imazethapyr and flumetsulam. A strip-split plot experiment was established at Lincoln University, Canterbury in April 2018. All cultivars established successfully with >110 seedlings/m^2. Herbicides were applied when the subterranean clover was at the 4-5 trifoliate leaf stage. Both herbicides increased the total subterranean clover yield for the season, with 'Napier' and 'Antas' being the highest yielding at ~6500 kg DM/ha. This was nearly double the yield of the lowest yielding cultivar, 'Trikkala'. This increase in subterranean clover yield was due to the reduction in competition due to both herbicides eliminating ~1000 kg DM/ha of broadleaf weeds. The cultivar*herbicide interaction at the first harvest demonstrates that the cultivars had different responses to the herbicide. Specifically, flumetsulam and imazethapyr increased the yield of 'Antas' and 'Napier'. 'Coolamon' yield was increased by flumetsulam but not by imazethapyr while the herbicides had no effect on the remaining cultivars at the first harvest.

The second part of this thesis investigated whether the *yanninicum* subspecies of subterranean clover is more suitable to be used in winter wet conditions in New Zealand.

An experiment was established at Lincoln University in July 2018. Two cultivars 'Monti', ssp. *yanninicum*, and 'Coolamon', ssp. *subterraneum*, were exposed to four watering treatments for eight weeks. 'Monti' was found to be more tolerant of waterlogging, having a 46% reduction in shoot dry weight in the waterlogged treatment compared with its highest yielding treatment. In contrast, 'Coolamon' was less tolerant of waterlogging with an 83% reduction in shoot dry weight compared with its highest yielding treatment. 'Coolamon' shoot dry weight was also reduced when watered 3x a week compared with 'Monti' which increased growth under the same treatment. The morphological strategy that allowed 'Monti' to be more tolerant to waterlogging appeared to be the production of lateral roots near and at the soil surface, which would allow the roots to absorb more oxygen. Photosynthetic rates decreased under waterlogging but the reduction was higher for 'Coolamon' than 'Monti' due to increased stomatal closure. 'Monti' produced anthocyanins in a response to waterlogging, showing that the plants were stressed, which may have provided an unknown protective factor. Eight weeks after treatments finished neither cultivar had recovered fully from waterlogging due to the previous effects on their root systems. This comparison has suggested that the ssp. *yanninicum* 'Monti' was more tolerant of waterlogging which suggests investigation of the impacts on other cultivars is warranted.

Keywords: Subterranean clover, *Trifolium subterraneum* L., ALS inhibiting herbicides, flumetsulam, imazethapyr, thermal time, canopy cover, broadleaf weeds, waterlogging, anthocyanins, lateral roots, photosynthesis, osmotic potential, relative water content, dryland pastures

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF EQUATIONS

LIST OF APPENDICES

1 GENERAL INTRODUCTION

Legumes are an important component of pastures in New Zealand due to their high nutritional value and ability to fix atmospheric nitrogen which improves soil quality and pasture production (Caradus *et al.*, 1995). New Zealand pastures are dominated by white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.). These pastures require high fertility and rainfall/irrigation but are not suited to summer dry, hill country areas (Monk *et al.*, 2016). White clover drought tolerance is low, due to a shallow root system, and post-drought recovery is slow leading to poor persistence in hill country areas (Knowles *et al.*, 2003). Subterranean clover (*Trifolium subterraneum* L.) is a more suitable legume choice as it avoids summer droughts by burying its seeds to allow regeneration the next year.

Subterranean clover is the most common annual clover used in New Zealand pastures (Monk *et al.*, 2016). There are three main subspecies of subterranean clover, *T. subterraneum* L. ssp. *subterraneum*, *T. subterraneum* L. ssp. *yanninicum* and *T. subterraneum* L. ssp. *brachycalycinum*, with all three having evolved in different environments (Katznelson, 1970; Smetham, 2003). Subterranean clover has high growth in early spring, compared with perennial options, which coincides with the high feed demand of lactating ewes (Brown *et al.*, 2006). Incorporating subterranean clover into a farming system has shown to increase pre-weaning lamb growth rates by ~30% and therefore increased prime lamb numbers (Grigg *et al.*, 2008). However, the use of subterranean clover by farmers in New Zealand is still low, partly due to an unreliable seed supply and no New Zealand bred cultivars (Monk *et al.*, 2016). Subterranean clover seed is grown and imported from Australia so cultivars are adapted to Australian conditions (Lucas *et al*., 2015). Seed is also often rejected for biosecurity reasons (e.g. soil contaminated seed) which means cultivar availability is variable.

During establishment subterranean clover is susceptible to being outcompeted by broadleaf weeds as is it sensitive to being shaded (Smetham, 2003). Therefore post emergent weed control is often necessary when establishing subterranean clover pastures and especially when establishing pure swards. However, there is only one herbicide, Headstart®, recommended for use on subterranean clover in New Zealand (Novachem, 2019). The majority of research on post-emergent herbicide use on subterranean clover has been conducted in Australia. Due to differences in climate and farming systems between the two countries New Zealand specific research is needed to provide local recommendations. There has been some previous research conducted in New Zealand with a limited number of cultivars (Lewis, 2017).

The most common subspecies grown in New Zealand is ssp. *subterraneum*. This subspecies is naturally found in free draining soils (Katznelson, 1970) which makes it intolerant to wet soils. Winter waterlogging can occur in summer dry areas of New Zealand, such as on mudstone soils on the North Island east coast.

1.1 Aims and Objectives

The aim of this thesis is to provide recommendations for herbicide use at establishment for subterranean clover cultivars in New Zealand and investigate whether ssp. *yanninicum* cultivars are more suitable than ssp. *subterraneum* for use in winter wet environments.

This thesis is structured in five chapters. Chapter 2 reviews the literature, outlining subterranean clover subspecies and cultivars in New Zealand, the current knowledge of acetolactate synthase (ALS) inhibiting herbicides for establishing subterranean clover and the mode of action of ALS inhibiting herbicides. The second half of the review covers subterranean clover subspecies responses to waterlogging and methods to quantify the response.

Chapter 3 deals with; Objective 1: to quantify the yield response of seven subterranean clover cultivars when sprayed with ALS inhibiting herbicides, Objective 2: to evaluate the visual effects of the herbicides on the subterranean clover cultivars and Objective 3: to document the impact of herbicides on common weeds of subterranean clover.

Chapter 4 deals with; Objective 4: to quantify the yield response of two subterranean clover cultivars, 'Coolamon' (ssp. *subterraneum*) and 'Monti' (ssp. *yanninicum*) under waterlogging, Objective 5: to identify the physiological and morphological mechanisms for any differences in response of subterranean clover cultivars to waterlogging and Objective

6: to quantify plant population and yield response of two ssp. *yanninicum* cultivars and two ssp. *subterraneum* cultivars sown together in a 50:50 mix under waterlogging.

Chapter 5 discusses the implication of these results in the context of a New Zealand dryland pasture system.

To investigate Objectives 1-3 a field experiment was conducted at Lincoln University. Pure swards of seven subterranean clover cultivars were sown and sprayed with two ALS inhibiting herbicides post emergence.

To investigate Objectives 4 and 5 four different watering treatments were applied to a ssp. *subterraneum* cultivar and a ssp. *yanninicum* cultivar to simulate waterlogging conditions. To investigate Objective 6 an area sown with two ssp. *subterraneum* and two ssp. *yanninicum* was flooded and their survival and growth responses quantified.

2 LITERATURE REVIEW

This literature review is comprised of three sections. Section 2.1 is a general overview of the subterranean clover subspecies and cultivars used in New Zealand. Section 2.2 discusses post-emergent herbicides for broadleaf weed control in subterranean clover. Section 2.3 covers subterranean clover tolerance to waterlogging along with common plant adaptations to waterlogging.

2.1 Subterranean clover

Subterranean clover is an annual legume that occurs naturally in areas with a Mediterranean climate (250-600 mm rainfall/year, hot, dry summers and moderate cool season temperatures) such as Spain, Portugal, Israel and Syria (Smetham, 2003; Nichols *et al.*, 2013b). It was first identified in New Zealand in in the early 1900s in Auckland and was first sown in Canterbury around the late 1920s (Saxby, 1956). Resident subterranean clover, 'Mt Barker', has been widespread on dry hill country across New Zealand since the 1980s (Suckling *et al.*, 1983). 'Tallarook' is naturalised in the North Island and 'Woogenellup' in the South Island around Blenheim (Richard Lucas, pers. comm. 22 October 2019).

There are three main subspecies of subterranean clover: *T. subterraneum* L. ssp. *subterraneum*, *T. subterraneum* L. ssp. *yanninicum* and *T. subterraneum* L. ssp. *brachycalycinum*. The natural distribution of these subspecies is based on edaphic factors (Smetham, 2003). Ssp. *subterraneum* is commonly found in acid-neutral, well drained soils, ssp. *yanninicum* in acidic, poorly drained soils and ssp. *brachycalycinum* in neutral-alkaline stony soils (Katznelson, 1970).

2.1.1 Life cycle

Subterranean clover seedling emergence occurs in autumn after rainfall. This is followed by vegetative growth throughout the winter and spring. Flowering occurs in spring. Flowering time is dependent on day length and temperature and differs among cultivars (Smetham, 2003). Subterranean clover plants die when conditions become dry in early summer so seeds are buried in a burr in the soil allowing the persistence of the species.

The seeds will then be summer dormant until they germinate the following autumn, when conditions are more suitable, which completes the life cycle (Smetham, 2003).

2.1.2 Cultivars

Subterranean clover cultivars historically or currently available in New Zealand are listed in Table 2.1. ‘Mt Barker’ and ‘Tallarook’ were widely sown in the 1940s and 50s and are still present in hill country pastures today (Chapman *et al.*, 1986; Lucas *et al.*, 2015). These cultivars were superseded by newer cultivars developed in Australia. Hardseededness is an important factor when selecting a cultivar for New Zealand conditions (Lucas *et al*., 2015). A hard seed coat prevents the seed from imbibing water, preventing ‘false strikes’ when the seed germinates too early in dry conditions that result in the death of the plant population in summer. Hardseededness is reduced over time and with summer-autumn temperature fluctuations (Smetham and Ying, 1991; Dodd *et al.*, 1995). Hardseededness reduces faster in Australia due to the higher soil temperatures so most cultivars sown in New Zealand typically have a low hardseededness rating (Table 2.1). This ensures that sufficient seed germinates the following autumn for successful establishment.

As with hardseededness, flowering date differs among cultivars (Nichols *et al*., 2013a). Early flowering cultivars are more suited to areas with a low rainfall and short growing seasons. Later flowering cultivars tend to have higher yields as the vegetative growth stage is longer (Smetham, 2003) but early spring growth can be slow making them susceptible to weed competition (Lucas *et al.*, 2015). Mid-late flowering cultivars are generally recommended in New Zealand (Smetham, 2003). Lucas *et al.* (2015) suggested sowing mixes of two complementary subterranean clover cultivars, e.g. mid vs late flowering or soft seeded vs hard seeded to cover site and seasonal variability.

One issue associated with the use of subterranean clover is its establishment. Traditionally subterranean clover is sown in autumn with a grass mix but the potential exists to establish and manage pure swards before drilling in grasses once a seed bank has been set. One aspect to consider in pure swards is the ability to control weeds.

Table 2.1 Subterranean clover culitvars historically or currently available in New Zealand with flowering times, hardseededness rating and burr burial rating from Nichols *et al*. (2013a).

Cultivar	Sub species	Flowering time	Hardseededness	Burr burial
'Antas'*	*Brachycalycinum*	Late	3	1
'Bindoon'	*Subterraneum*	Early	3	7
'Campeda'	*Subterraneum*	Early	5	6
'Coolamon'*	*Subterraneum*	Mid	5	7
'Denmark'*	*Subterraneum*	Late	2	5
'Karridale'	*Subterraneum*	Late	2	6
'Leura'	*Subterraneum*	Late	2	5
'Monti'*	*Yanninicum*	Early	2	6
'Mt Barker'	*Subterraneum*	Late	1	3
'Napier'*	*Yanninicum*	Late	5	6
'Narrikup'*	*Subterraneum*	Mid	3	7
'Rosabrook'	*Subterraneum*	Late	5	6
'Tallarook'	*Subterraneum*	Late	1	5
'Trikkala'*	*Yanninicum*	Early	2	6
'Woogenellup'	*Subterraneum*	Mid	1	3

Hardseededness: 1 least hard, 10 most hard. Burr burial: 1 little or no burial, 9 strong burr burial. *cultivars used in Experiment 1.

2.2 Herbicides

Herbicides can be selective or non-selective. Non-selective herbicides kill/damage all plants whereas selective herbicides kill/damage the weeds and ideally have minimal impact on the crop (Cobb and Reade, 2010). Selectivity is achieved by targeting physiological differences between the crop and weed but is relative and dependent on the dose, i.e. a large dose of herbicide will likely impact the crop as well as the weeds (Cobb and Reade, 2010). There are several classes of herbicides, which are categorised by their mode of action. This section focuses on acetolactate synthase (ALS) inhibiting herbicides as they have previously been shown to be the most tolerated by subterranean clover (Lewis, 2017) and thus have potential to be used if establishing pure swards.

2.2.1 Subterranean clover tolerance to post emergence herbicides

Most herbicide manuals give a tolerance for 'clover' which usually refers to white clover (Novachem, 2019). Indeed, there has been limited research carried out in New Zealand on subterranean clover herbicide tolerance with most research being conducted in Australia (Bowran, 1993; Dear *et al.*, 1995; Gilmour, 1996). However, Lewis (2017) investigated the effects of eight herbicides on four subterranean clover cultivars ('Antas', 'Denmark', 'Monti' and 'Narrikup') at Lincoln University, Canterbury, New Zealand. Herbicides were applied at the 1-2 and 4-6 trifoliate leaf stage on 14 June and 12 July 2016, respectively. Phytotoxic responses and yield were measured.

Imazethapyr and flumetsulam were found to be the most tolerated herbicides by the subterranean clovers when applied at the 1-2 trifoliate leaf stage (Lewis, 2017). Imazethapyr and flumetsulam increased the total season subterranean clover yield of 'Narrikup' and had no effect on the other cultivars. Imazethapyr treated 'Narrikup' had the highest sown clover yields of 2600 kg DM/ha. The 2,4-DB decreased the subterranean clover yield of 'Antas' but not the other cultivars. When applied at the 4-6 trifoliate leaf stage, imazethapyr, flumetsulam and bentazone had no effect on total season subterranean clover yield, which ranged between 420-600 kg DM/ha. Subterranean clover yields decreased in MCPB, 2,4-DB and bromoxynil + diflufenican treatments compared with the control. Subterranean clover yields were higher when herbicide was applied at the 1-2 trifoliate leaf stage due to greater control of broadleaf weeds than when herbicide application was delayed to the 4+ trifoliate leaf stage. Lewis (2017) suggested delayed spraying in mid-winter may not be worthwhile as there was no yield increase and reproductive success may be reduced if subterranean clover densities decrease.

When applied at the 1-2 trifolate leaf stage flumetsulam, imazethapyr and bromoxynil + diflufenican were the most effective herbicides. They reduced the broadleaf weeds by over 83% compared with the control. Imazethapyr and flumetsulam were also the most effective at controlling the broadleaf weeds when applied at the 4+ trifoliate leaf stage, along with bentazone. However, the control was not as effective as the earlier application with the total broadleaf weed yield being reduced by 75% compared with the control, likely due to a larger weed size at the time of spraying.

Lewis (2017) concluded that flumetsulam, imazethapyr and bentazone were the best broadleaf herbicides for use on subterranean clover, but that bentazone would have less effective weed control in cold conditions likely to occur during a New Zealand autumn when subterranean clover pastures are being established. Therefore, flumetsulam and imazethapyr were used in Experiment 1.

2.2.2 ALS inhibiting herbicides

Since their introduction in the 1980s, acetolactate synthase (ALS) inhibiting herbicides have become one of the most widely used herbicides for weed control (Zhou *et al.*, 2007). This is due to their low toxicity to animals, which do not possess the ALS enzyme, and their high toxicity to plants which is approximately 100 times more potent than herbicides used pre-1980s (Whitcomb, 1999). This allows low rates of herbicides to be applied in g/ha instead of kg/ha. There are five classes of ALS herbicides; imidazolinones, triazolopyrimidines, pyrimidinylthio-benzoates, sulfonylureas, and sulfonylamino-carbonyltriazolinones (Cobb and Reade, 2010). All five classes have the same mode of action but are chemically different.

2.2.2.1 Imazethapyr

Imazethapyr is part of the imidazolinones herbicide class. It is sold in New Zealand as Spinnaker® (240 g/L of active ingredient (a.i.)) by BASF New Zealand Ltd. It is recommended for the post emergence control of weeds in 'clover' seed crops and lucerne but which clover that is, is not specified (BASF, 2016).The recommended rate for clover crops is 0.4 L/ha and can be applied from when the clover has two trifoliate leaves. A follow up spray for grass control may be necessary (Novachem, 2019). Spinnaker® is not currently recommended for subterranean clover in New Zealand. In Western Australia, Spinnaker® (700 g/kg imazethapyr) is recommended for post emergence weed control in subterranean clover and can be applied from the three trifoliate leaf stage (BASF, 2012).

Table 2.2 Spinnaker® recommended rates and weeds controlled (BASF, 2016).

Rate (L/ha)	g a.i./ha	Weeds controlled
0.3-0.4	72-96	Chickweed, cleavers, hedge mustard
0.4	96	Annual poa, catsear, chamomile, chickweed dandelion, dock, doves foot, fathen, fennel, field madder, groundsel, henbit, mallows, nightshade, shepherds purse, sorrel, spurrey, storksbill, twin cress, willow weed, wireweed, yarrow and Yorkshire fog

Previous research has shown mixed results on subterranean clovers tolerance to imazethapyr. Dear and Sandral (1999) found imazethapyr caused no leaf burn when applied at a rate of 43 and 72 g a.i./ha on cultivars 'Trikkala' and 'Karridale' 30 days after treatment (DAT). Imazethapyr also had no effect on the yields of 'Trikkala' after 30 days. However, 'Karridale' yield was reduced at 30 DAT but had recovered by 90 DAT. There was no effect on seed yield. In a glasshouse experiment, there was also no effect on 'Trikkala' yield 30 DAT when Spinnaker® was applied at a rate of 0.25 L/ha (Dear *et al.*, 2006). However, Evers *et al.* (1993) found a reduction in yield of the cultivar 'Clare' from 940 kg DM/ha to 340 kg DM/ha three months after application of imazethapyr at a rate of 0.7 kg/ha, in Texas. The subterranean clover had 13% visible injury 37 DAT which increased to 22% 72 DAT. The experiment was repeated the following year. There was no reduction in yield and the visible clover injury was reduced to 7% at 39 DAT. In the second year, the harvest was taken 20 DAT which may not have given the clover sufficient time to respond, as the cool temperatures led to slow growth.

The imazethapyr tolerance of a wide range of cultivars ('Urana', 'Coolamon', 'Dalkeith', 'York', 'Napier', 'Gosse' and 'Riverina') was investigated in a field trial in NSW, Australia (Sandral and Dear, 2005). Imazethapyr was applied at the rate of 72 g a.i./ha at the 3-4 trifoliate leaf stage. In the first year, 'York' had a decrease in yield of 76%. There was no effect on the other cultivars. The following year only 'Urana', 'Napier' and 'Dalkeith' were unaffected by the application of imazethapyr. 'Coolamon' had the largest reduction in yield of 93%. The remaining three cultivars had a reduction in yield ranging from 57-63%.

2.2.2.2 Flumetsulam

Flumetsulam is part of the triazolopyrimidines herbicide class. Headstart® is a flumetsulam herbicide distributed in New Zealand by Lonza NZ Ltd. Headstart® contains 50 g/L of flumetsulam as the active ingredient in the form of an oil dispersion (Lonza, 2018). Headstart® is recommended for the control of broadleaf weeds while maintaining selectivity to clover, lucerne, chicory and grass pasture. As with Spinnaker®, Headstart® can be applied from two trifoliate leaves onwards and at several rates depending on weeds present (Table 2.3). It is not recommended to apply in cold and/or wet conditions. Headstart® is currently the only herbicide recommended for subterranean clover use in New Zealand (Novachem, 2019).

Table 2.3 Headstart® recommended rates, weeds controlled and application times (Lonza, 2018).

Rate (L/ha)	Rate (g a.i./ha)	Weeds controlled	Application time
0.5	25	Chickweed, spurrey, wild radish, hedge mustard	before 4th leaf
0.8	40	Amaranthus, broad-leaved dock, cleaver, black nightshade, fathen, inkweed, shepards purse, storkbill, twincress, mallow	before 4th leaf
		annual buttercup, creeping yellow cress, yellow gromwell	before flowering
1.0	50	Dandelion, hawkbit, scrambling speedwell	before 4th leaf
		Stinking mayweed, field madder, field pansy, wireweed, soreel	seedling stage
		giant buttercup	before flowering
		nodding thistle	before 6th leaf

There is limited literature on the effects of flumetsulam on subterranean clover especially on newer cultivars. In Western Australia, flumetsulam was applied to two subterranean cultivars, 'Dalkeith' and 'Nungarin', at a rate of 40 g/ha at the 3-5 trifoliate leaf stage (Bowran, 1993). Yield was scored at flowering. Both cultivars had a 27% reduction in yield compared with the control. Another trial in Western Australia, used Broadstrike (800 g a.i/kg flumetsulam) at a rate of 25 g/ha to control doublegee (*Emex australis*) and capeweed (*Arctotheca calendula*) in subterranean clover seed crops (Gilmour, 1996).

Flumetsulam treated clover had higher seed yields in three out of five sites studied. The other two sites had no change from the control. Regeneration of the subterranean clover was also measured. Flumetsulam increased the seedling population from 150 seedlings/m^2 in the control to 240 seedlings/m^2.

2.2.2.3 Mode of action

ALS inhibiting herbicides work by inhibiting the ALS enzyme which is part of the pathway for producing three branched-chained amino acids (BCAAs): valine, leucine and isoleucine (Zhou *et al.*, 2007; Cobb and Reade, 2010). ALS is an enzyme found in the chloroplast of higher plants. ALS is the catalyst in the first step of two pathways of BCAA synthesis, with one producing valine and leucine and the other producing isoleucine (Zhou *et al.*, 2007). For valine and leucine, 2-acetolactate is synthesised from two pyruvate molecules. For isoleucine, 2-acetohydroxybutyrate is synthesised from 2-ketobutyrate and pyruvate. These acetohydroxy acids undergo further synthesis to produce the BCAAs. ALS inhibitors bind to the ALS enzyme entry site which prevents molecules, such as pyruvate, entering the enzymes active site and preventing the reaction.

It is not fully understood why plants die following treatment with ALS inhibiting herbicides (Zhou *et al.*, 2007; Cobb and Reade, 2010). Protein synthesis decreases due to ALS inhibition as the cell is deficient in BCAAs (Zhou *et al.*, 2007). This slows down the rate of cell division which results in cell death. The effects of ALS inhibiting herbicides are first seen in young shoots and roots as amino acid synthesis primarily occurs in young tissue (Singh and Shaner, 1995). Growth is inhibited within hours of the herbicide application but phytotoxicity symptoms are not seen for several days (Cobb and Reade, 2010). Chlorosis and necrosis are seen in the meristem tissue which is followed by wilting of young leaves and then the entire plant. Plant death starts to occur from 10 days but can take up to two months depending on temperature. Secondary effects of ALS inhibition such as the accumulation of toxic intermediate compounds and reduction of respiration have also been identified and may also contribute to plant death (Zhou *et al.*, 2007).

ALS inhibiting herbicides are selective, targeting the weed species while leaving the crop unharmed. Species selectivity is due to rates of metabolism of the herbicide, with faster rates in the tolerant plant, rather than differences in herbicide uptake or movement (Cobb and Reade, 2010). Small chemical changes within herbicide classes and families can change selectivity and potency (Ladner, 1990). Imazethapyr and flumetsulam belong to different herbicide classes and have different structures (Figure 2.1). Therefore they may differ in selectivity for weeds and potentially for cultivars of subterranean clover.

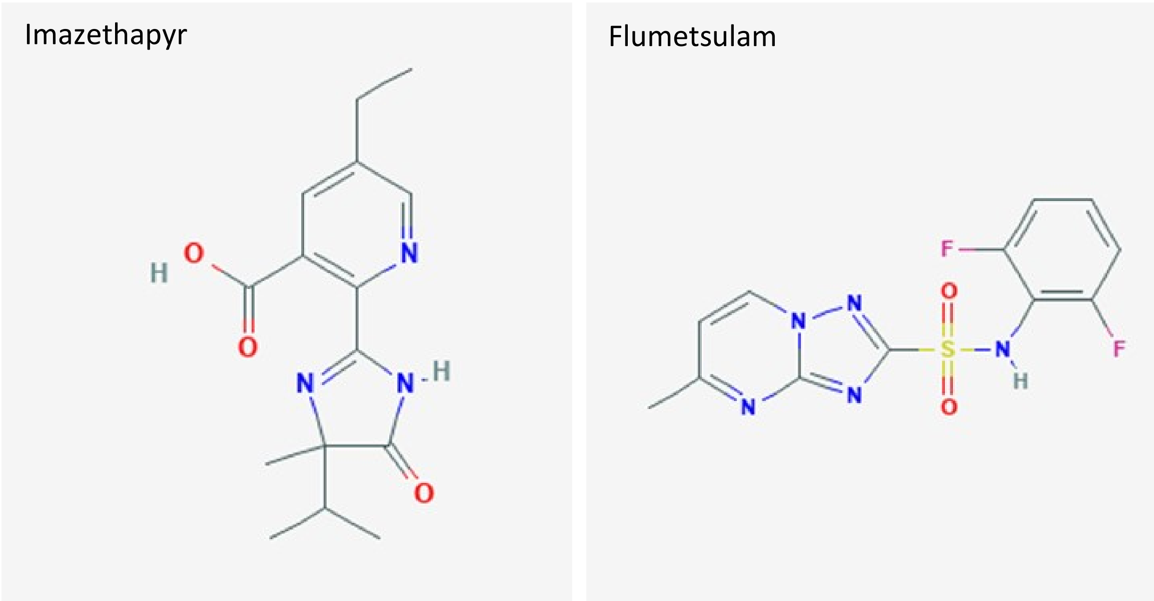

Figure 2.1 Chemical structures for imazethapyr (PubChem, 2005a) and flumetsulam (PubChem, 2005b).

2.2.2.4 White clover tolerance to ALS herbicides

More research has been conducted on white clover ALS herbicide tolerance than subterranean clover. Phytotoxicity of white clover seedlings to imazethapyr was investigated in a greenhouse experiment (Adami *et al.*, 2017). Imazethapyr was applied at three rates, 100, 150 and 200 g a.i./ha, when the white clover was at the three trifoliate leaf stage and phytotoxicity measured on a scale of 0 (normal plants) – 100% (total plant death). The lowest rate of imazethapyr resulted in no phytotoxic symptoms (Table 2.4). The two higher rates of imazethapyr showed the highest phytotoxicity levels of 20% on days 21 and 28 after application before levels declined back to the control 49 days after application.

Table 2.4 Herbicide phytotoxic levels (%) on white clover for treatments applied at the three-trifoliate leaf growth stage. Adapted from Adami *et al.* (2017).

Rate of imazethapyr (g a.i./ha)	Days after application						
	7	14	21	28	35	42	49
Control	0_b	0_b	0_b	0_b	0_b	0_b	0
100	0_b	0_b	0_b	0_b	0_b	0_b	0
150	10_a	15_a	20_a	20_a	13_a	10_a	0
200	10_a	15_a	20_a	20_a	18_a	15_a	5

Imazethapyr has been shown to have a slight negative effect on white clover. When applied to mature white clover imazethapyr (70 g a.i./ha) gave 10% control of white clover (100%=complete plant death) when assessed six weeks after treatment (McCurdy *et al.*, 2013). White clover height was also reduced by 45%. In another experiment imazethapyr increased white clover cover when applied to an established white clover pasture (Enloe *et al.*, 2014). White clover cover increased from 49% to 67% 60 days after imazethapyr was applied at 100 g a.i./ha in winter due to control of broadleaf weeds, which declined from 27% to 1% cover in the pasture. Imazethapyr may have some temporary negative effects on the individual white clover plants (McCurdy *et al.*, 2013; Adami *et al.*, 2017). However, when applied to a pasture with a high content of broadleaf weeds (Enloe *et al.*, 2014) any negative effect is likely to be negated by the decrease in competition, and therefore increased growth of white clover, due to the control of broadleaf weeds.

Flumetsulam is also generally tolerated by white clover. Flumetsulam applied to established pastures found white clover was suppressed at <6% at rates of 25 and 50 g a.i./ha 1-2 months after application (Harris and Husband, 1997). When applied at 100 g a.i./ha white clover was suppressed by 18%. However, 80% control of weeds was achieved with the 50 g a.i./ha rate so higher rates resulting in larger damage to white clover are unnecessary. Cold, wet weather following herbicide applications resulted in higher levels of white clover yellowing, due to slower plant metabolism. Yellowing disappeared within a week of warmer weather. Flumetsulam is also able to be used while establishing white clover pastures. Flumetsulam was applied to a mixed sward of white and red clover (*Trifolium pratense* L.), narrow-leaved plantain (*Plantago lanceolata* L.) and chicory

(*Cichorium intybus* L.) when plants were at the 2-4 leaf growth stage (Gawn *et al.*, 2012). There was little phytotoxic effect on the clovers at the highest rate of flumetsulam (52 g a.i./ha) with a score of 8 (10 = healthy plant) four weeks after treatment and a score of 7.8 32 weeks after treatment. The dry matter of the clovers increased from 3 kg DM/ha in the control to 69 kg DM/ha seven weeks after treatment due to the reduction in competition from the broadleaf weeds. Chicory dry matter was also increased by flumetsulam. Plantain dry matter was reduced seven weeks after treatments from 88 kg DM/ha to 11 kg DM/ha but had recovered by the end of the trial, 32 weeks after application. This suppression of plantain may be beneficial as plantain can often outcompete clover during establishment (Gawn *et al*., 2012).

White clover is often present in the soil seed bank of New Zealand pasture. Therefore, white clover in Experiment 1 was also examined for herbicide tolerance.

2.2.2.5 Soil residue

Imazethapyr has been found to persist for at least three years and flumetsulam for at least two years depending on soil conditions (Hollaway *et al.*, 2006b). Flumetsulam (20 g a.i./ha) and imazethapyr (72 g a.i./ha) were applied at several sites with different soil types to monitor leaching and persistence in the soil. Ten months after application an average of 29% of applied imazethapyr was present in the top 40 cm of soil. Imazethapyr initially degraded quickly, followed by a slow degradation phase with low concentrations persisting in the clay soil types three years after application (Figure 2.2). However, imazethapyr degraded faster in the sandy soil at Mount Hope, South Australia. Canola yield was reduced up to 24 months after treatments in the clay soils but there was no reduction in yield 12 months after treatment in the sandy soil (Hollaway *et al.*, 2006a). Degradation rate of imazethapyr increases with both increasing soil moisture and temperature (Goetz *et al.*, 1990). However, variation at the degradation rate between sites was likely due to soil type, as rainfall, temperature and pH were similar between sites (Hollaway *et al.*, 2006b).

Flumetsulam was less persistent in the soil than imazethapyr. Ten months after application an average of 10% of applied flumetsulam amount was present in the top 40 cm of soil, with undetectable amounts at two of the five sites (Hollaway *et al.*, 2006a). Temperature has a significant impact on flumetsulam degradation with the half-life decreasing from 88 days to 30 days when incubation temperature increased from 15°C to 30°C (McDowell *et al.*, 1997). Flumetsulam degradation may also be influenced by organic matter, with Shaw and Murphy (1997) finding flumetsulam persistence decreased with decreasing organic matter. This is supported by McDowell *et al.* (1996), who found a slight decrease in flumetsulam half-life in low organic matter soil, although this might have also been influenced by differing pH between the low and high organic matter soil. Other studies suggest pH has no effect on flumetsulam persistence (Shaw and Murphy, 1997).

Material removed due to copyright compliance

Figure 2.2 Imazethapyr residues detected by bioassay in 0–40 cm soil profile following application in 3 consecutive years at Dooen, Kaniva and Mount Hope. Lines show the fitted models for each site (Hollaway *et al.*, 2006b).

Residuals of the two herbicides used in Experiment 1 were monitored by quantifying the weed populations the following autumn after spraying.

2.2.2.6 Resistance

ALS inhibiting herbicide resistance has developed surprisingly quickly. ALS inhibiting herbicides have the highest number of resistant weed populations compared with the other herbicide classes, even though they were only commercialised in 1982 (Tranel and Wright, 2002). Herbicide resistance has occurred by two mechanisms, 1: reduced sensitivity of the ALS enzyme to the herbicide and 2: increased metabolism to rapidly detoxify the herbicide. ALS herbicide resistance can be developed by as little as a single amino acid change within the ALS enzyme which can help explain why resistance has developed so quickly (Zhou *et al.*, 2007). Resistance was first observed in Kansas in 1987 in a population of prickly lettuce (*Lactuca serriola* L.) that was resistant to sulfonylurea herbcides and some imidazolinones, including imazethapyr, where sulfonylureas herbicides had been used for the previous five years (Mallory-Smith *et al.*, 1990). Common sunflowers (*Helianthus annuus* L.) became resistant to imazethapyr after seven years of application (Al-Khatib *et al.*, 1998). Resistance of some broadleaf weeds, sow thistle (*Sonchus oleraceus* L.) and Indian hedge mustard (*Sisymbrium orientale* L.), to imazethapyr and flumetsulam has also been reported in Australia (Boutsalis and Powles, 1995). To prevent resistance it is recommended to use a herbicide rotation with different modes of actions (BASF, 2016; Lonza, 2018). However, since it has been previously shown that other modes of action are harmful to subterranean clover (Lewis, 2017) this may be difficult to achieve and could lead to future problems. However, in a pastoral sense this should be less of a problem because the herbicide would only be used once in the establishment phase. This contrasts an annual cropping or subterranean clover seed crop situation where consecutive annual applications could be expected.

2.3 Waterlogging

Subterranean clover is grown in summer dry areas with a low annual rainfall. However, some areas that are dry in summer may have high rainfall/waterlogging in winter and early spring which could impact the growth of subterranean clover. Previous research has suggested the ssp. *yanninicum* is more tolerant to wet conditions than ssp. *subterraneum* (Francis and Devitt, 1969; Cocks, 1994). However, no research had been conducted in New Zealand conditions to examine this aspect.

Plant yield decreases under waterlogged conditions, unless the plant can adapt. Waterlogging results in the changes of several soil characteristics. The most important is the reduction in the amount of oxygen available to the roots, as gases in soil pores are replaced by water. Soil redox potential declines under waterlogged conditions which affects the availability of plant nutrients (Parent *et al.*, 2008). An increase in soil pH is also associated with waterlogging. When soils are hypoxic (low in oxygen) respiration is decreased due to limited oxygen. In anoxic soils (no oxygen) anaerobic respiration occurs. Anaerobic respiration requires fermentation to supply the NAD^+ needed for glycolysis and the production of ATP (Taiz and Zeiger, 2006). Glycolysis efficiency is reduced when under anaerobic conditions so an increased rate of glycolysis is required to provide the cells with adequate ATP. This leads to the depletion of carbohydrate reserves.

2.3.1 Subterranean clover yield response to waterlogging

As the three subspecies of subterranean clover are adapted to different environments (Katznelson, 1970; Smetham, 2003) they are likely to have differing tolerances to waterlogging. For example, subterranean clover seedlings from the three subspecies, *subterraneum, yanninicum* and *brachycalycinum*, were flooded to 25 mm above the soil level, six weeks after emergence by Francis and Devitt (1969). Each subspecies was represented by 25 cultivars. Shoots and roots were harvested 21 days after flooding began. Shoot dry matter production of ssp. *yanninicum* was not affected by the flooding treatment. However, ssp. *subterraneum* and *brachycalycinum* shoot yield were reduced by 26% and 46% respectively, compared with the control. Root growth decreased in response to waterlogging for all three subspecies. Ssp. *brachycalycinum* had the greatest reduction in root growth of 76% and ssp. *yanninicum* was the most tolerant, with root growth reducing by 26%. Ssp. *subterraneum* root growth reduced by 46%. Surface roots developed in some flooded ssp. *yanninicum* cultivars, but were not present in any ssp. *subterraneum* or *brachycalycinum* cultivars, which could be a reason for the increased waterlogging tolerance.

Rogers and West (1993) also found shoot yield decreased by 22% in ssp. *brachycalycinum* ('Clare') after being flooded 10 mm above the soil surface for 15 days. New root development was decreased by 50% under waterlogging.

In contrast to Francis and Devitt (1969), waterlogging did not reduce the shoot dry weight of ssp. *subterraneum* ('Dalkeith') when immersed in water for 34 days (Gibberd and Cocks, 1997). Plants were grown in pots in a glasshouse and submerged two months after emergence. However, root dry weight was reduced with 40% of the root system dying within 12 days of waterlogging. It seems likely that if waterlogging had continued for longer there would have been a decrease in shoot growth due to the reduction in roots. Gibberd *et al.* (2001) also found no reduction in shoot dry weight for either spp. *subterraneum* ('Dalkeith') or ssp. *yanninicum* ('Trikkala'). Subterranean clover grown in pots was flooded to 5 mm above the soil surface for 35 days. Relative growth rate was not affected by the waterlogging treatment for either subspecies but root:shoot ratio decreased by 25% compared with the control for ssp. *subterraneum.* There was no change in root:shoot ratio for ssp. *yanninicum*. A reason for the conflicting results for ssp. *subterraneum* between the experiments may be due to only one cultivar being used whereas (Francis and Devitt, 1969) used 25 ssp. *subterraneum* cultivars. 'Dalkeith' may be more tolerant to waterlogging than other ssp. *subterraneum* cultivars.

Ssp. *brachycalycinum* is the least tolerant to waterlogging which is not surprising given its adaptation to stony soils. Ssp. *subterraneum* may be tolerant to waterlogging but the literature is conflicting. Ssp. *yanninicum* is likely the most tolerant to waterlogging due to its ability to maintain roots when waterlogged. Ssp. *yanninicum* has been found to be more resistant to root rot than the other two subspecies which may be a contributing factor to its tolerance (Flett *et al.*, 1993). Therefore, in this study the only commercially available ssp. *yanninicum* cultivar 'Monti' was compared with a subterranean subspecies to see if any evidence of a difference in tolerance was observed.

2.3.2 Root mechanisms

There are two main mechanisms by which roots adapt to waterlogged soils (Armstrong *et al.*, 1991). The first is the production of lateral roots on or near the soil surface. The second is an increase in root porosity by aerenchyma formation.

2.3.2.1 Lateral root formation

Waterlogged soils are low in oxygen, with surface layers being aerobic and deeper layers being anaerobic. Plants can adapt to this by producing lateral roots, near the soil surface or even above the soil (Armstrong *et al.*, 1991). Lateral roots are often thin to increase surface area available to absorb oxygen. Increased production of lateral roots is usually associated with lower shoot growth. Surface roots have been seen in some ssp. *yanninicum* cultivars but this was not associated with an increase in root dry matter (Francis and Devitt, 1969). Lateral root production has also been observed in other *Trifolium* species. The total root length of Persian clover (*Trifolium resupinatum* L.) increased after 34 days of waterlogging by 40% compared with the control, due to the production of lateral roots (Gibberd and Cocks, 1997). Balansa clover (*Trifolium michelianum* Savi.) also produces lateral roots in response to waterlogging (Rogers and West, 1993).

Root dry matter was obtained from Experiment 2 to compare the production of lateral roots of the two subspecies.

2.3.2.2 Aerenchyma formation

Aerenchyma are gas-filled channels that form in the root and facilitate gas exchange between the roots and shoots (Taiz and Zeiger, 2006). There are two types of aerenchyma, lysigenous and schizogenous. Lysigenous aerenchyma is formed through the death of cells in the root cortex which leaves a gas-filled space (Evans, 2004). Hypoxia is one of the initiators of lysigenous aerenchyma. Hypoxia causes the root tip to produce more ethylene, from the stimulation of 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase, which results in the death of root cortex cells (Taiz and Zeiger, 2006). Schizogenous aerenchyma is formed by cells separating rather than dying and is common in wetland plants (Evans, 2004). This process is a part of normal development and is not usually influenced by environmental factors such as hypoxia. Increasing aerenchyma during waterlogging allows the root system to obtain oxygen from the atmosphere, via the shoots. Aerenchyma also allows the transport of gases needed for nitrogen fixation to the root nodules of legumes (James *et al.*, 1992; Pugh *et al.*, 1995).

Lysigenous and schizogenous aerenchyma are both formed by subterranean clover spp. *subterraneum* and *yanninicum* (Gibberd et al., 2001). Ssp. *yanninicum* has predominately lysigenous aerenchyma and ssp. *subterraneum* has predominately schizogenous aerenchyma. Both sub species increased their root porosity when grown in a hypoxic solution for 35 days but ssp *yanninicum* had a higher root porosity of 13.8% compared with 9.6% for ssp. *subterraneum* (Plate 2.1). Subterranean clover cultivar 'Clare' (ssp. *brachycalycinum*) had no increase in root porosity after waterlogging for 15 days compared with white clover which doubled root porosity in new roots as a response to waterlogging (Rogers and West, 1993).

Material removed due to copyright compliance

Plate 2.1 Transverse section of lateral roots from subterranean clover ssp. *yanninicum* (A) and ssp. *subterraneum* (B) after growing in a hypoxic solution for 35 days (Gibberd *et al.*, 2001).

2.3.3 Nitrogen fixation

Legumes can fix atmospheric nitrogen through the symbiotic relationship with *Rhizobium* bacteria (Taiz and Zeiger, 2006). Nitrogen fixation occurs in nodules on the roots formed by the rhizobia. Nodules are a pinky-red colour when nitrogen fixation is occurring due to leghemoglobin, an oxygen binding protein. Both white and subterranean clovers can fix ~28 kg N/t DM of clover (Lucas *et al.*, 2010).

There is no literature on the effect of waterlogging on subterranean clover nitrogen fixation but this has been studied in white clover. White clover ('Katrina') grown in pots was submerged in water up to the soil surface for nine weeks from germination (Pugh *et al.*, 1995). Waterlogging had no effect on nitrogenase activity but vacuole volume increased in cells in the nodules of waterlogged clover from 1.25×10^{-3} to 6.03×10^{-3} μm^3. White clover that had been normally watered was then flooded for 24 hours. Nitrogenase activity was reduced by 96% after 24 hours of waterlogging from white clover that had been previously watered normally. This suggests that white clover can adapt to waterlogging if waterlogged from germination. Larger aerenchyma had developed in roots of white clover waterlogged from germination which allowed the transport of gases to the nodules. The increased vacuoles in the nodule cell may result in oxygen becoming more available to the *rhizobia* due an increase in surface area of the cells.

The potential for nitrogen fixation can be estimated by scoring nodule size and colour (Peoples *et al.*, 1989). As leghemoglobin is pinky-red nodule colour increases in redness with nitrogen fixation which can be visually scored. Therefore, the nodule colour and size was examined from roots in Experiement 2.

2.3.4 Anthocyanins

Anthocyanins are red and purple water soluble pigments found in a wide variety of plant species (Chalker-Scott, 1999). Foliar anthocyanins may be permanent or produced to protect the plant from a range of environmental factors including temperature, drought or anoxia. Photosynthetic rates can decrease with production of anthocyanins as they absorb blue light and reflect red. They therefore compete with chlorophyll for light (Chalker-Scott, 2002).

Subterranean clover leaf reddening has previously been observed in New Zealand when plants were exposed to cold temperatures (Teixeira *et al.*, 2019). In that situation, cultivars from the three subterranean clover subspecies were exposed to cold temperatures for two weeks during winter. Cultivars from the ssp. *yanninicum* had greater leaf redness, which averaged 83% leaf redness across three cultivars, compared with ssp. *subterraneum*, which averaged 29% leaf redness across 10 cultivars. The only ssp. *brachycalycinum* cultivar 'Antas' had 20% leaf redness. Leaf redness had no effect on plant yield. Leaf reddening has also been observed in subterranean clover as a response to virus (Harvey and Harvey, 2009) and root rot (Wong *et al.*, 1986).

Leaf reddening in response to waterlogging has been shown to occur in seedlings of several tree species (Chalker-Scott, 2002). Waterlogged soils are low in oxygen which can result in the deterioration of roots and reduction of water uptake. This can cause the shoots of the plant to become water stressed. The production of anthocyanins increases the solutes in the leaf cell which helps the leaf maintain tolerable water conditions even when waterlogged.

2.3.5 Photosynthesis

Photosynthesis is the process by which water and carbon dioxide are transformed by light energy into carbohydrates and oxygen (Taiz and Zeiger, 2006). There was no change in photosynthetic rate for white clover that had been waterlogged for eight days (Blaikie *et al.*, 1988) but reductions in photosynthesis due to waterlogging have occurred in perennial ryegrass (McFarlane *et al.*, 2003) and wheat (*Triticum aestivum* L) (Malik *et al.*, 2001). Reductions in photosynthetic rate in waterlogged plants may be due to decreased stomatal conductance which reduces the amount of CO_2 absorbed. Another factor may be the accumulation of carbohydrates in the leaves, due to the slow growth rate of waterlogged plants that may cause a feedback inhibition of photosynthesis (Malik *et al.*, 2001).

2.3.6 Plant water relationships

Plant water potential is an indicator of overall plant health and is used as a measure of the water status of the plant (Taiz and Zeiger, 2006). Water potential (Ψ_w) is influenced by three factors; osmotic (or solute) potential (Ψ_s), hydrostatic pressure (Ψ_p) and gravity (Ψ_g) (Equation 2.1). Osmotic potential is a measure of the amount of dissolved solutes in the water. Positive hydrostatic pressure raises water potential and is referred to as turgor pressure. Gravity has little impact on water potential at a cellular level and is therefore generally omitted from the equation. Plants require a lower water potential than the soil to uptake water.

Equation 2.1 $$\Psi_w = \Psi_s + \Psi_p + \Psi_g$$

2.3.6.1 Osmotic adjustment

Osmotic adjustment is the process in which cells accumulate solutes to decrease water potential. This is a common physiological response to drought which has been shown to occur in a wide variety of plants including perennial ryegrass (Thomas, 1986; Taiz and Zeiger, 2006). Osmotic adjustment can also occur as a response to waterlogging, although there is limited information in the literature and none for subterranean clover. Osmotic potential decreased from -0.85 MPa to -1.35 MPa in castor bean (*Ricinus communis* L.) after waterlogging for 15 days (Gadallah, 1995). Osmotic potential has also been shown to decrease in tomato plants (*Solanum lycopersicum* L.), although not as much as plants exposed to drought (Seng, 2014).

2.3.6.2 Relative water content

The water content of the leaf tissue can also be used as an indicator of plant water status (Ehlers and Goss, 2003). Relative water content (RWC) is a measure of the water content of the leaf cells compared with the water content of the cells at full turgidity. Generally, well-watered plants have a RWC of 88% or greater at midday (Hsiao, 1990). When RWC drops below this the plant becomes wilted and photosynthesis is reduced. Irreversible cell damage and death occurs when RWC is in the range of 50-60% for several hours. As RWC is a direct measurement of cell hydration and relative volume it is consistent among species

2.4 Conclusions

ALS herbicides have been shown to be the least damaging to subterranean clover but there is limited data available for New Zealand conditions, with most research occurring in Australia. The application of two ALS herbicides, flumetsulam and imazethapyr, typically cause a small or no reduction in yield. However, this appears to be dependent on cultivar and may also be impacted by other factors such as temperature so results can differ between years. Therefore, this thesis adds to research previously undertaken in New Zealand to increase our understanding of weed control in subterranean clover.

Waterlogging decreases the yield of subterranean clover. Ssp. *yanninicum* may be more tolerant of waterlogging than ssp. *subterraneum* but the literature is inconclusive. This is probably due to the ability of ssp. *yanninicum* to maintain root mass under waterlogged conditions, but could also be due to physiological mechanism, such as photosynthesis, which have not yet been investigated for subterranean clover. Currently, there is a lack of field studies on herbicide and waterlogging tolerance with cultivars that are available in and grown in New Zealand.

3 HERBICIDE EXPERIMENT

3.1 Introduction

Post-emergent herbicides can aid the successful establishment of subterranean clover. Weed control during autumn, when subterranean clover is germinating, has been shown to be necessary to prevent clover seedlings from being outcompeted by the faster growing broadleaf weeds (Evers *et al.*, 1993). Previous research on subterranean clover in New Zealand has shown ALS inhibiting herbicides are the most tolerated by subterranean clover, while also giving effective broadleaf weed control (Lewis, 2017).

The three subterranean clover subspecies are suited to different environmental conditions (Smetham, 2003). Therefore cultivars from all three subspecies were used in this experiment. 'Coolamon', 'Denmark' and 'Narrikup' were the three ssp. *subterraneum* used and are all commercially available in New Zealand. Of the three ssp. *yanninicum* cultivars used only 'Monti' is commercially available in New Zealand. 'Napier' and 'Trikkala' are not currently available but were included to have a wider range of ssp. *yanninicums*. 'Antas' is the only commercially available ssp. *brachycalycinium* and was therefore included.

Experiment 1 aimed to provide recommendations for herbicide use at establishment for subterranean clover cultivars in New Zealand. To do this there were three main objectives:

Objective 1: To quantify the yield response of seven subterranean clover cultivars when sprayed with ALS inhibiting herbicides.

Objective 2: To evaluate the visual effects of the herbicides on the subterranean clover cultivars.

Objective 3: To document the impact of herbicides on common weeds of subterranean clover.

3.2 Materials and Methods

3.2.1 Germination tests

Germination tests were performed in laboratory conditions to confirm seed viability of the cultivars sown (Table 3.1). Seeds were incubated at 16°C, which is the optimal temperature for subterranean clover germination (Moot *et al.*, 2000). Seeds were said to be germinated when the radicle was twice the length of the seed.

Table 3.1 Thousand seed weight (TSW), germination, hard seed percentage and field emergence for all subterranean clover cultivars used in Experiment 1 at Iversen 9, Lincoln University, Canterbury, New Zealand.

Cultivar	TSW (g)	Germination (%)	Hard seed (%)	Emergence rate (%)
'Antas'	13.2	97	1.3	96
'Coolamon'	6.82	91	8.0	55
'Denmark'	7.64	90	5.3	68
'Monti'	10.2	79	12	84
'Napier' coated	29.4	91	6.7	96
'Napier' uncoated	12.6			
'Narrikup'	9.08	89	1.3	58
'Trikkala'	10.7	89	5.3	67

3.2.2 Iversen 9

Experiment 1 was sown at the north end of Iversen 9 field (43.6473°S, 172.4667°E), at Lincoln University, Canterbury, New Zealand on the 20 April 2018. The soil at the site is classified as a Wakanui silt loam (Udic Ustochrept, USDA Soil taxonomy). A soil sample at the depth of 0-75 mm was taken on 24 April 2018 and analysed by Hill Laboratories, Hamilton, New Zealand. The results of these are summarised in Table 3.2. The soil test results indicate a low amount of available sulphur. The subterranean clover had yellowing leaves, which were thought to be signs of a sulphur deficiency, by late August 2018. Yellow and green leaf samples from 'Antas' were taken and analysed for nutrient deficiencies. Both green and yellow leaves were deficient in sulphur (Table 3.3). Therefore Sulphur Super 30 (0,7,0,30) was applied to the plots on 11 September 2018 at a rate of 332 kg/ha, or the equivalent of 100 kg S/ha.

Table 3.2 Soil test (0-75 mm) for the north end of Iversen 9, Lincoln University, Canterbury, New Zealand on 20 April 2018

Analysis	Iversen 9
pH	6.1
Potentially available N (kg/ha)	79
Olsen P (mg/L)	22
Potassium (me/100g)	0.67
Sulphate S (mg/kg)	5

Table 3.3 Leaf nutrient values for 'Antas' subterranean clover on 24 August 2018 from I9, Lincoln University, Canterbury, New Zealand. Standard critical values for N, P, K and S in white clover and sub clover from literature sources. Adapted from Olykan *et al.* (2019).

Nutrient	I9 subterranean clover		Marginal deficiency[1]		Optimum[2]
	Green leaf	Yellow leaf	Sub clover	White clover	White clover
N	4.13	3.04	3.0–3.2	4.4–4.7	4.8–5.5
P (mg/kg)	0.32	0.30	0.30–0.40	0.30–0.34	0.35–0.40
K (mg/kg)	1.36	1.47	1.50–2.50	1.7–1.9	2.0–2.4
S (mg/kg)	0.23	0.18	0.18–0.30	0.22–0.26	0.27–0.32

[1.] Associated with a reduction in plant growth but no visible signs of deficiency (Reuter and Robinson, 1997).

[2.] For samples of 'leaf ' plus (part) petiole taken from grazing height under conditions conducive to active growth (Cornforth, 1984).

3.2.3 Paddock History

The experimental area came out of a pasture of 'Arrow' perennial ryegrass sown at 20 kg/ha and 'Tribute' white clover at 4 kg/ha sown on 26 August 2014 . On 2 March 2018 the area was sprayed with WeedMaster TS540 (540 g/L glyphosate) at 2 litres/ha. On 26 March 2018 the area was rotary hoed and then rolled two days later. Following this on 19 April 2018 the area was power harrowed and then rolled into a final seed bed a day prior to sowing the experiment.

Weeds in I9 were surveyed after the experiment had been sown and are detailed in Table 3.4. The dominant weed species were broad leaved dock and wire weed.

Table 3.4 Common and botanical names of weeds found in Iversen 9, Lincoln University, Canterbury, New Zealand.

Common name	Botanical name
Annual poa	*Poa annua* L.
Broad leaved dock	*Rumex obtusifolius* L.
Chickweed	*Stellaria media* L.
Hedge mustard	*Sisymbrium officinale* L.
Scrambling speedwell	*Veronica persica* L.
Shepherd's purse	*Capsella bursa-pastoris* L.
Spurrey	*Spergula arvensis* L.
Twin cress	*Lepidium didymium* L.
Wireweed	*Polygonum aviculare* L.

3.2.4 Climate data

Monthly rainfall data for April 2018-May 2019, and the long term mean, are presented in Figure 3.1. Long term means were obtained from the Broadfields meteorological station (43°62'S, 172°47'E) from the NIWA CliFlo database. In April 2018, when the experiment was sown, rainfall was 115 mm or 70 mm higher than the long term mean. This trend of higher than average rainfall continued in May and June. In July and August 2018 rainfall was 25 mm and 36 mm lower than average. Spring (September-November) 2018 had higher rainfall than average, notably in November which received 130 mm or over double the average rainfall. December also had 15 mm above average rainfall. The remaining summer months were consistent with the long term mean.

Mean air temperature for April 2018-May 2019 was consistent with the long term means, with the largest difference of +1.5°C occurring in July 2018 and March 2019 (Figure 3.2).

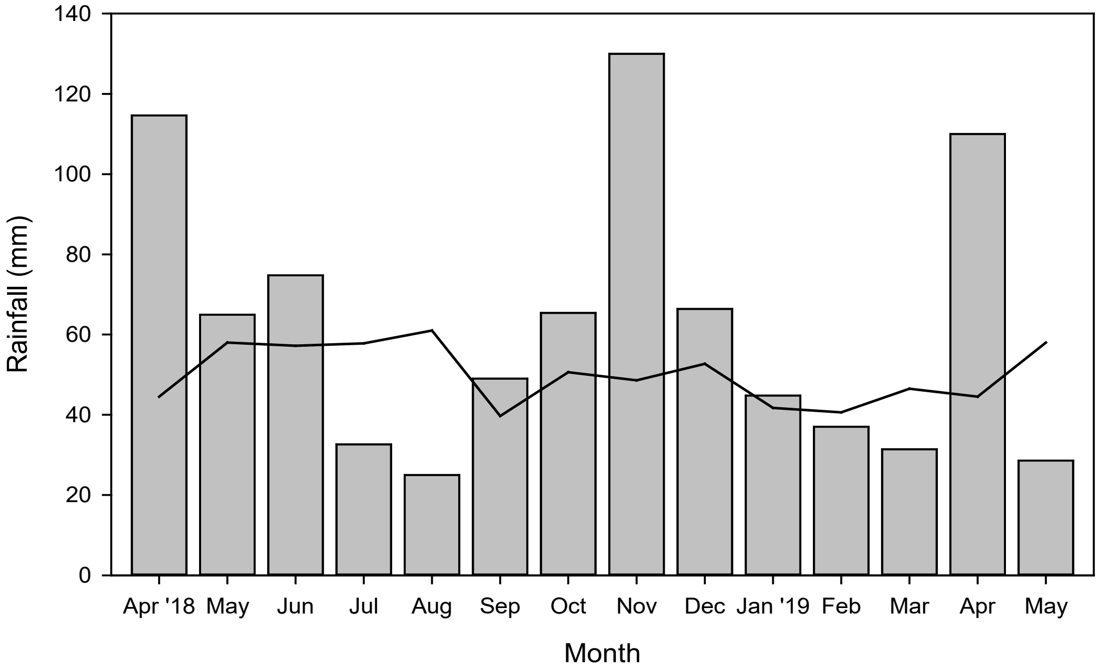

Figure 3.1 Monthly rainfall (mm) (■) from April 2018-May 2019 from the I7 meteorological station, Lincoln University and the long term mean (1981-2010) (–) from Broadfields Meteorological Station, Lincoln, Canterbury, New Zealand.

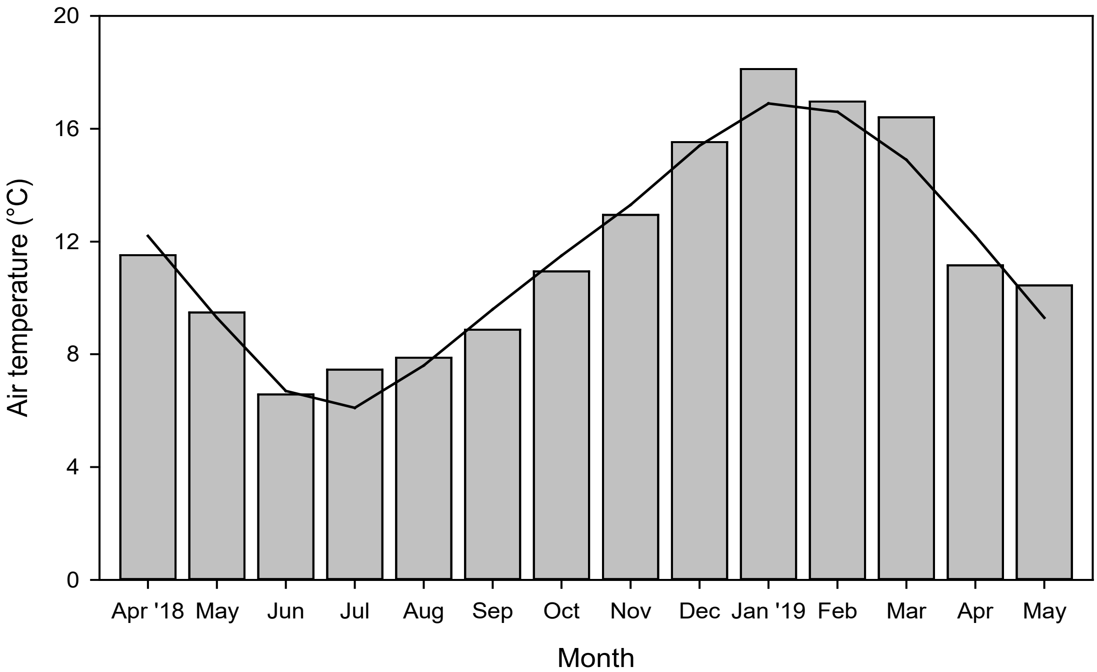

Figure 3.2 Mean monthly air temperature (°C) (■) from April 2018-May 2019 from the I7 meteorological station, Lincoln University and the long term mean (1981-2010) (–) from Broadfields Meteorological Station, Lincoln, Canterbury, New Zealand.

3.2.5 Experimental Design

Seven subterranean clover cultivars across three different sub species were used in Experiment 1 (Table 3.5). Cultivars were sown at an above commercial sowing rate of 20 kg/ha. ‘Napier’ was sown at a higher rate of 40 kg/ha to account for the lime coated seed which represent ~50% of the weight. This was determined by the removal of the lime coat followed by weighing to determine the TSW of the uncoated seed (Table 3.1). The cultivars were sown with a Flexiseeder 14 row air induced plot seeder on the 20 April 2018 at a 15 cm row width. The coulters were chisel tip with press wheels for depth control and a spring tensioning system for downwards pressure on each coulter. The plots were 16 x 4.2 m and arranged in a randomized block with four replicates. Two herbicide treatments were applied across the plots creating a strip-split plot design. The herbicide treatments were applied in a 2 m wide strip. The unsprayed control strips were 3 m wide. The headlands around the plot were sown on 24 April 2018 with a Fiona drill at 10 kg/ha of a ssp. *yanninicum* mix (‘Napier’ and ‘Trikkala’) and 10 kg/ha of a ssp. *subterraneum* mix (‘Denmark’ and ‘Narrikup’). The Fiona drill had a flax coulter setup that drilled 75 cm rows. Emergence occurred from 1 May 2018.

After each dry matter harvest, plots were grazed with mixed aged ewes from 4-8 October, 5-7 November and 10-12 December 2018. The plots were topped following grazing on 9 October 2018 to remove dead weeds that had not been grazed. This was predominantly necessary because of the weed invasion in the control plots and may not have been necessary commercially after spraying.

Table 3.5 Subterranean clover cultivars, subspecies and sowing rate used in Experiment 1 at Iversen 9, Lincoln University, Canterbury, New Zealand.

Cultivar	Sub species	Sowing rate (kg/ha)
‘Antas’	*Brachycalycinum*	20
‘Coolamon’	*Subterraneum*	20
‘Denmark’	*Subterraneum*	20
‘Monti’	*Yanninicum*	20
‘Napier’*	*Yanninicum*	40
‘Narrikup’	*Subterraneum*	20
‘Trikkala’	*Yanninicum*	20

* = coated seed, = 17 kg/ha of bare seed equivalent.

3.2.6 Herbicide Application

The herbicide treatments (Table 3.6) were applied on the 04 July 2018 when all subterranean clover cultivars were at the 4-5 trifoliate leaf stage. Commercially recommended application rates were used and mixed in 10 L batches. Headstart® was applied with 1 L/ha of 'Uptake' spraying oil. Herbicides were applied with a purpose built sulky sprayer with TeeJet fan nozzle boom with 2.1 m spray cover. Herbicides were applied at walking speed on a still day.

Table 3.6 Herbicide application rates used in Experiment 1 at Iversen 9, Lincoln University, Canterbury, New Zealand.

Herbicide	Active ingredient	Application rate (L/ha)
Headstart®	50 g/L flumetsulam	1.0
Spinnaker®	240 g/L imazethapyr	0.4

3.2.7 Measurements

3.2.7.1 Seedling establishment

Prior to herbicide application, two 0.3 m sections of drill row were marked out in each cultivar plot. Seedling counts of subterranean clover were taken on 13 and 21 June 2018. After the herbicide application, on 4 July 2018, a 0.5 m section of drill row was marked out in the centre of each herbicide plot. Seedling counts were taken the day of application (04 July 2018) and then on 19 July 2018 to determine any seedling death from the herbicide. After this individual plants were difficult to count accurately. On 4 September 2018 a 20 cm length of drill row was excavated from each plot and the number of seedlings was counted.

3.2.7.2 Dry Matter Production

Dry matter measurements were taken four times during the experiment. The first sample was in association with the 0.2 m length of drill row excavated from each plot on 4 September 2018. For the next three harvests, 3 October, 4 November, 6 December 2018, a 0.2 m^2 quadrat area was cut at 2.5 cm above the soil surface. A subsample was then sorted into subterranean clover, white clover, broadleaf weeds, grass weeds and dead components. The samples were dried in a forced air oven at 60^{o}C for 48 hours before weighing. For the final harvest, broadleaf weed samples from the cultivar 'Monti' were sorted into weed species to assess the impact of herbicide on individual broadleaf weed species.

3.2.7.3 Thermal Time

Thermal time was used to investigate the relationship between subterranean clover yield and temperature and to determine the temperature adjusted growth rate (TAGR). Data from the Broadfields meteorological station was used to calculate daily thermal time values. Thermal time can be calculated using Equation 3.1 where Tt is thermal time, T_{max} is the maximum daily temperature, T_{min} is the minimum daily temperature and T_b is the base temperature.

Equation 3.1 $$\mathbf{Tt\ (°Cd)} = \frac{T_{max} - T_{min}}{2} - T_b$$

For this experiment, thermal time was calculated using the Jones and Kiniry (1986) method where a sinusoidal function is fitted to mean daily air temperatures, at three hourly intervals, excluding periods where T_{min} is less than T_b. A base temperature of 0°C was used as it has previously been identified as a base temperature for other annual clovers (Monks, 2009; Nori, 2013). Calculations were repeated with a base temperature of 3°C so comparisons could be made to pasture (Mills, 2007).

3.2.7.4 Canopy cover

Normalised Difference Vegetation Index (NDVI) of the plot was measured using a Trimble GreenSeeker Handheld Crop Sensor. The GreenSeeker emits bursts of red and infra-red light and then measures the amount of light reflected back from the plants to determine NDVI. NDVI for individual plots was measured by holding down the GreenSeeker trigger, resulting in continuous measurements, and walking across the plot. The GreenSeeker would then display an average of the continuous measurements taken. The GreenSeeker was held perpendicular to the ground roughly a metre high. Starting on 7 September 2018, measurements were taken about every 10-20 days until 6 December 2018.

Equation 3.2, from Oliveira (2015), was used to correct NDVI measurements from the GreenSeeker where $NDVI_r$ is the Index measured, $NDVI_s$ is the bare soil Index measurement, C_{max} is the actual maximum radiation interceptance (0 = no canopy, 1 = fully covered) and $NDVI_{max}$ is the highest NDVI reading for the plot throughout the season. C_{max} was considered to be 0.95. $NDVI_{corrected}$ could then be multiplied by 100 to get canopy cover %.

Equation 3.2

$$\boldsymbol{NDVI_{corrected}} = \frac{(\boldsymbol{NDVI_r} - \boldsymbol{NDVI_s})(\boldsymbol{C}_{\mathbf{max}})}{(\boldsymbol{NDVI_{max}} - \boldsymbol{NDVI_s}}$$

3.2.7.5 Phytotoxicity Assessment

The European Weed Research Society (EWRS) phytotoxicity damage score was used to assess the impact of herbicides on the subterranean clover (Table 3.7). A visual assessment of the subterranean clover was taken seven days after herbicide application and then weekly for the first two weeks and then fortnightly until 26 September 2018. Whole plots were scored. Clover plants in the herbicide treatments were scored relative to the unsprayed control plants which by definition had an EWRS score of 1.

Table 3.7 European Weed Research Society (EWRS) phytotoxicity damage score used to assess herbicide damage of subterranean clover at Iversen 9, Lincoln University, Canterbury, New Zealand.

Score	Description of effects
1	No damage/healthy plant
2	Very mild symptoms
3	Mild but clearly recognisable symptoms
4	More severe symptoms but no effect on yield
5	Reduction in yield expected, thinning, severe chlorosis, leaf burn or suppression
6	
7	Above commercial threshold, severe damage
8	
9	Plant death

3.2.7.6 Emergence: Autumn 2019

Subterranean clover emergence the following autumn was also monitored. Seedling counts were taken on the 21 February 2019. Sub clover seedlings were counted in a 0.01 m^2 quadrat in each plot. Dry areas and areas that were dominant in white clover were avoided. The plots were then sprayed with 5 L/ha of glufosinate-ammonium (Buster, 200 g a.i./L) to kill all seedlings to quantify any further emergence. By March 2019 it was too difficult to count individual seedlings and plots were visually assessed using a 0-6 scale (Table 3.8) adapted from Teixeira *et al.* (2018). Plots were scored as a cultivar as there were no notable differences among herbicide treatments and seedling numbers were high.

Table 3.8 Subterranean clover emergence scale used in Experiment 1 at Iversen 9, Lincoln University, Canterbury, New Zealand. Adapted from Teixeira *et al.* (2018).

Score	Seedlings/0.01m^2	Seedlings/m^2
0-1	2.5	250
2	5.7	570
3	8.9	890
4	12	1200
5	15	1520
6	>20	>2000

3.2.7.7 Dock control 2019

In early 2019 dock was observed to be the dominant weed species. The residual effect of the herbicide treatments was measured by scoring the amount of observable docks in the herbicide plots on 15 March 2019. A 0-5 scale was used, with 0 equalling no docks.

3.2.8 Statistical Analysis

All results were analysed using Genstat 19th edition. Two-way split-strip plot ANOVAs were used to analyse; seedling establishment (post herbicide treatments), dry matter production, botanical composition, EWRS phytotoxicity scores and daily growth rates. The treatment structure was set as cultivar*herbicide. Reps were used for blocking, herbicide treatment for rows and cultivar for columns. Means were separated using Fisher's protected least significant difference (LSD) with a significance level of α=0.05.

Botanical composition data were transformed using an arcsine transformation as proportions did not fit in 0.0-0.3, 0.3-0.6, 0.6-1.0 groups (Sokal and Rohlf, 1981). LSDs and SEMs were then back transformed, for presentation in the results section.

A one way ANOVA was used for seedling establishment (pre-herbicide treatments), broadleaf weed components for 'Monti' at the final harvest, emergence scores in autumn 2019 and the residual dock control. Reps were used for blocking and means were separated using Fisher's protected LSD with a significance level of α=0.05.

A linear regression with groups of accumulated DM yield and accumulated thermal time was performed to determine TAGR. A one way ANOVA, with reps used for blocking, was then used for TAGR and x-axis intercept. Means were separated using Fisher's protected LSD with a significance level of α=0.05.

3.3 Results

3.3.1 Seedling establishment

'Denmark' had the highest (P=0.009) seedling population of 185 plants/m^2 on the 11 June 2018 (Figure 3.3). The remaining six cultivars averaged 127 plants/m^2. A week and a half later seedling population peaked and there was no difference (P=0.104) among the cultivars, which ranged between 210 and 400 plants/m^2.

On the day the herbicide treatments were applied, 'Denmark' (178 plants/m^2), 'Coolamon' (175 plants/m^2) and 'Napier' (153 plants/m^2) had the highest (P=0.008) seedling populations. 'Monti' and 'Antas' had the lowest number of ~100 plants/m^2. On the 19 July there was no difference (P=0.078), with populations remaining relatively stable compared with the previous measurement. At the final seedling count, on 4 September 2018, 'Denmark' and 'Coolamon' had the highest (P<0.001) seedling populations of 250 and 230 plants/m^2 respectively. 'Monti' and 'Antas' had fewer than half the seedling population of 'Denmark', averaging 110 plants/m^2.

The herbicide treatments had no effect on the number of seedlings on either the 19 July (P=0.886) or 4 August (P=0.335).

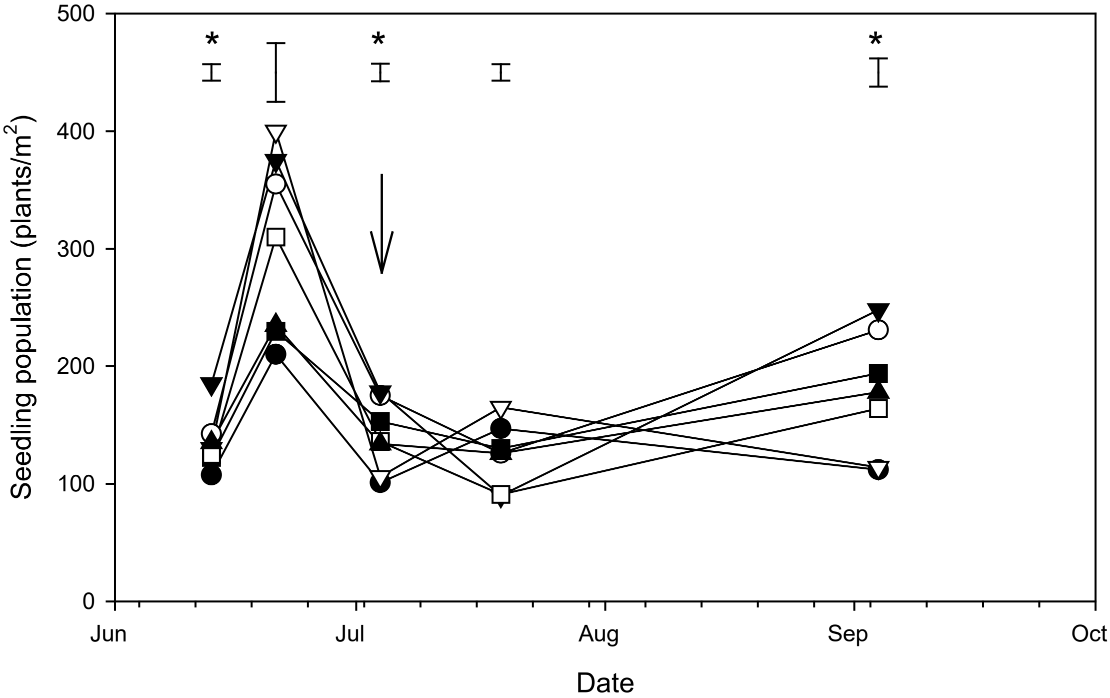

Figure 3.3 Seedling establishment of seven subterranean clover cultivars; 'Antas' (●), 'Coolamon' (○), Denmark (▼), 'Monti' (▽), 'Napier' (■), 'Narrikup' (□), 'Trikkala' (▲) at Iversen 9, Lincoln University, Canterbury, New Zealand. Error bars are SEM for the main effect of cultivar. * = main effect of cultivar is significant (P<0.05). Arrow indicates the application of herbicide.

3.3.2 Dry matter production

Unless otherwise stated there were no interactions between herbicide and cultivar for the measured variables.

3.3.2.1 Seedling harvest: 4 September 2018

The results of the subterranean clover yield from the seedling harvest on 4 September 2018, 137 days after sowing, are shown in Figure 3.4. There was no herbicide effect (P=0.110) on the yield of subterranean clover. However, 'Coolamon', 'Denmark' and 'Napier' had higher (P=0.043) yields, ranging from 1750-2000 kg DM/ha, than 'Narrikup', which averaged 1110 kg DM/ha. The remaining cultivars ranged between 1360-1520 kg DM/ha.

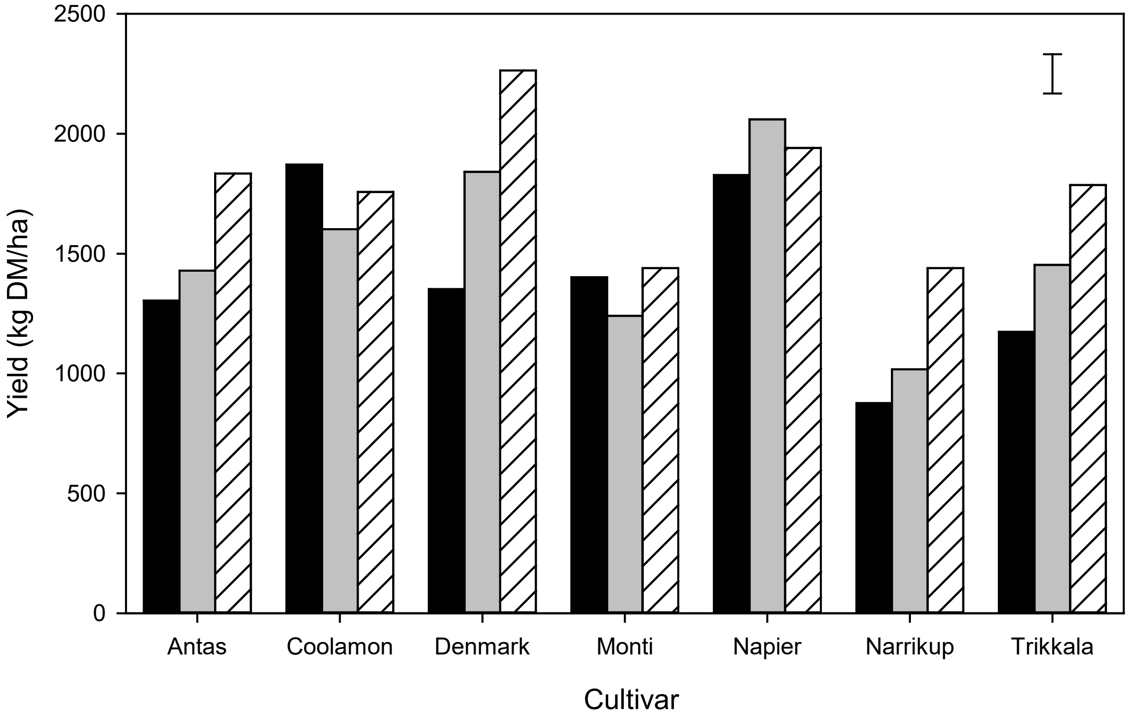

Figure 3.4 Subterranean clover dry matter (DM) yield of seven cultivars on 4 September 2018, after treatment with herbicides at establishment, at Iversen 9, Lincoln University, Canterbury, New Zealand. Control (■), flumetsulam (■), imazethapyr (▨). Error bar is the SEM for main effect of cultivar.

3.3.2.2 Total dry matter yield: 1 May 2018-6 December 2018

Over the course of the experiment, the accumulated total dry matter yields were highest (P=0.003) from 'Napier' (8350 kg DM/ha) and 'Antas' (8160 kg DM/ha), shown in Figure 3.5. There was no difference in total dry matter for the remaining five cultivars. These ranged from 6260-6880 kg DM/ha. Herbicide had no effect (P=0.288) on total yield.

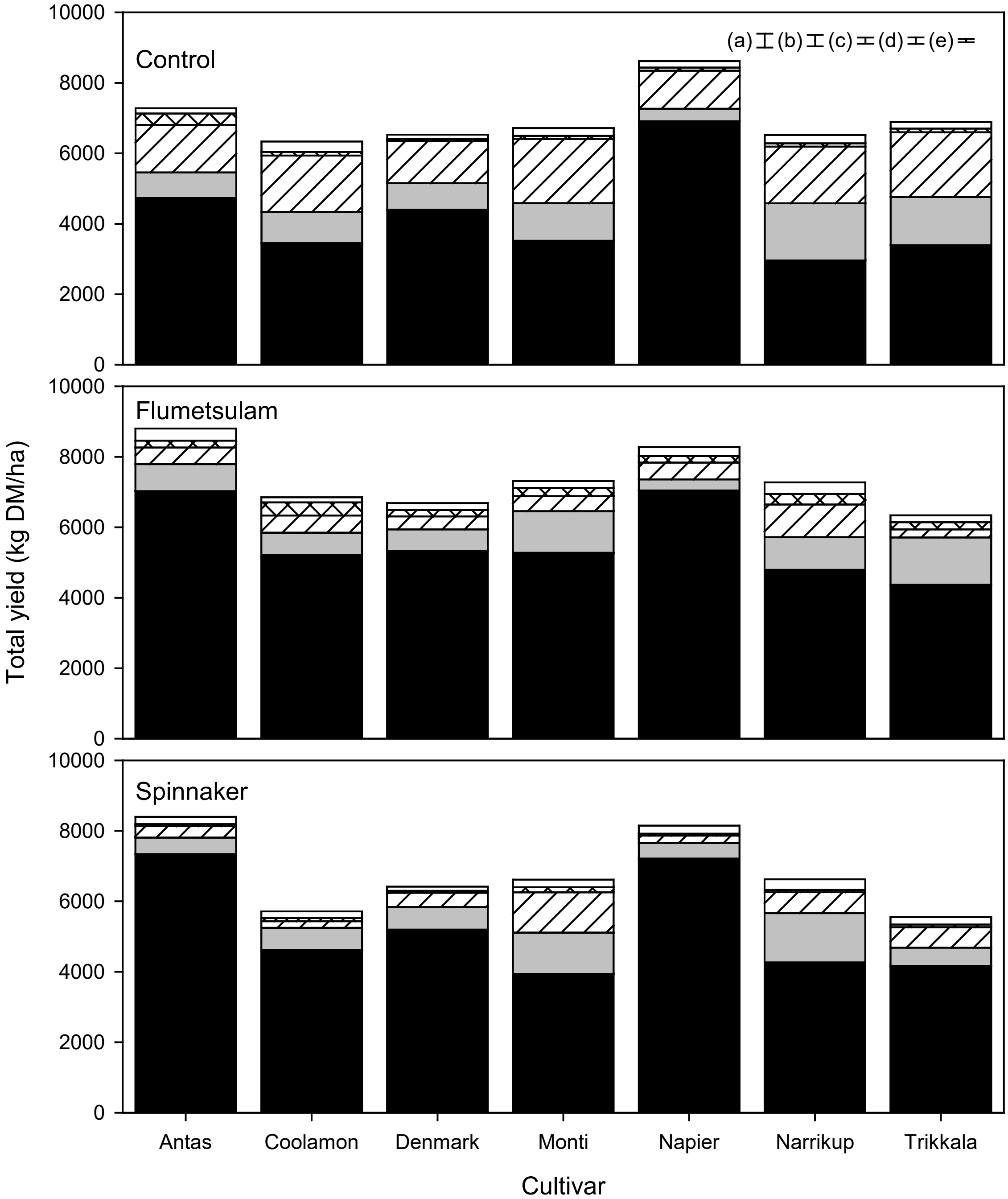

Figure 3.5 Total dry matter (DM) yield of subterranean clover cultivars, from 1 May 2018 to 6 December 2018, after treatment with herbicides at establishment, at Iversen 9, Lincoln University, Canterbury, New Zealand. Categories are subterranean clover (■), white clover (■), broadleaf weed (▨), grass weed (⊠) and dead matter (□). Error bars are the SEM for (a) the main effect of cultivar for total DM; (b) the main effect of cultivar for subterranean clover DM; (c) the main effect of herbicide for subterranean clover DM; (d) the main effect of cultivar on white clover DM, (e) the main effect of herbicide for broadleaf weed DM.

However, when the composition of the total dry matter was considered there were differences. The subterranean clover yield was higher (P=0.004) in the flumetsulam (5620 kg DM/ha) and imazethapyr (5330 kg DM/ha) treatments than the control (4220 kg DM/ha). Cultivar also had an effect (P<0.001) on subterranean clover yield. 'Napier' and 'Antas' had the highest subterranean clover yields (7050 and 6370 kg DM/ha, respectively), which was 3000 kg DM/ha higher than the lowest subterranean clover yielding cultivar, 'Trikkala'.

White clover yield was highest (P=0.024) in 'Narrikup' (1320 kg DM/ha), 'Monti' (1140 kg DM/ha) and 'Trikkala' (1070 kg DM/ha), compared with 370 kg DM/ha in 'Napier'.

Broadleaf weed yield was reduced (P<0.001) to ~480 kg DM/ha in both the herbicide treatments compared with 1450 kg DM/ha in the control. Grass weed yield was not affected (P=0.106) by herbicide treatments and averaged 150 kg DM/ha. Dead matter was also unaffected (P=0.754) by herbicide treatment or cultivar and averaged 220 kg DM/ha.

3.3.2.3 Harvest 1: 3 October 2018

To examine the temporal pattern of growth, the yields and botanical composition from individual harvests were also examined.

At the first harvest on 3 October 2018, total dry matter yield was affected (P=0.005) by the herbicide*cultivar interaction (Figure 3.6). Flumetsulam treated 'Antas' had the highest total dry matter yield of 2830 kg DM/ha, which was 600 kg DM/ha greater than its control. Imazethapyr reduced the total dry matter yield of 'Coolamon' by 670 kg DM/ha compared with the control. Both herbicide treatments (Imazethapyr 950 kg DM/ha, Flumetsulam 1280 kg DM/ha) reduced the total dry matter yield of 'Denmark' compared with the control (1950 kg DM/ha). The total yield of all other cultivars was unaffected by herbicide treatment.

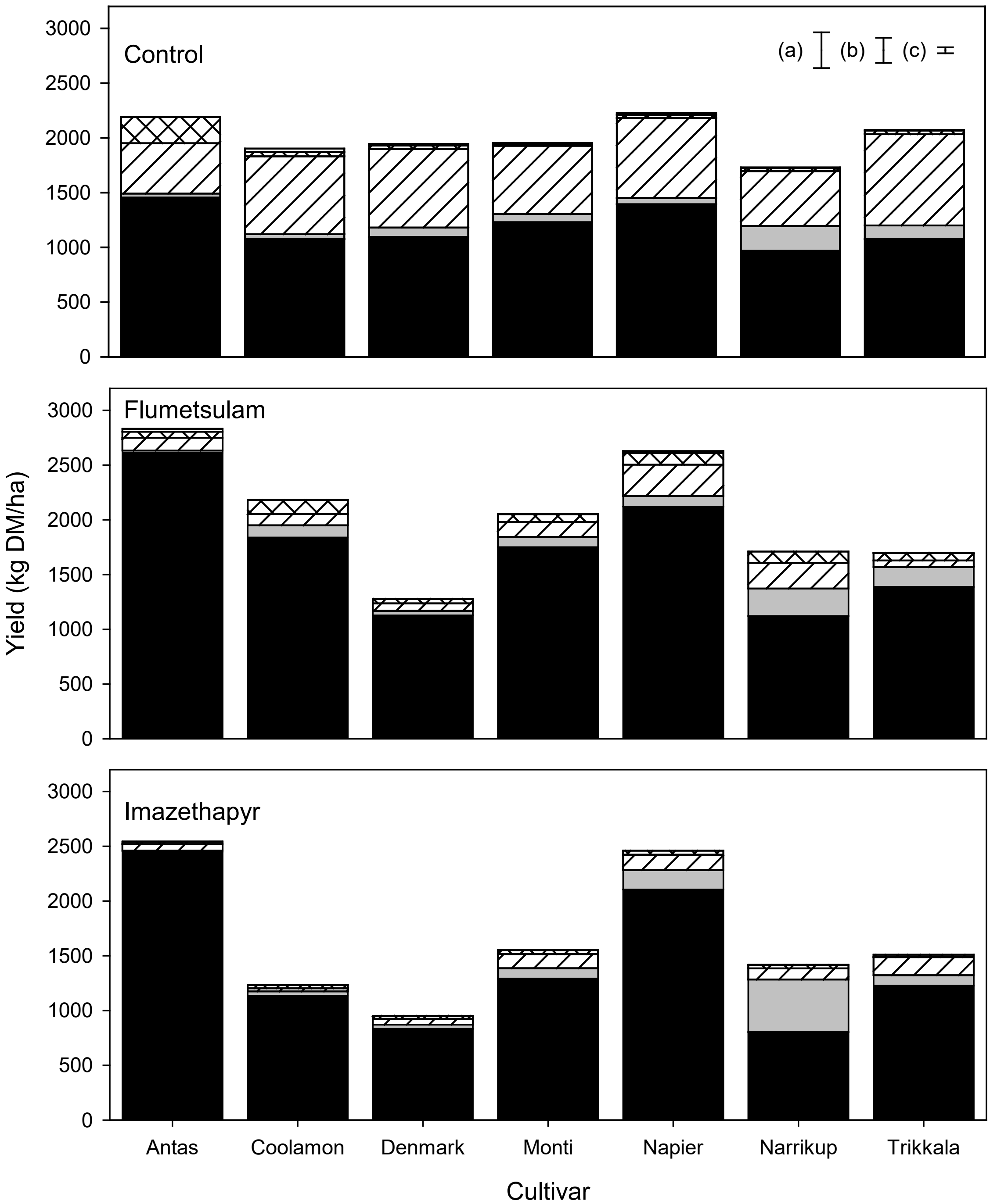

Figure 3.6 Dry matter (DM) yield of subterranean clover cultivars on 3 October 2018 after treatment with herbicides at establishment, at Iversen 9, Lincoln University, Canterbury, New Zealand. Categories are subterranean clover (■), white clover (■), broadleaf weed (▨), grass weed (⊠) and dead matter (□).Error bars are the SEM for (a) cultivar*herbicide interaction for total DM; (b) cultivar*herbicide interaction for subterranean clover DM; (c) the main effect of herbicide for broadleaf weed DM.

There was also a herbicide*cultivar interaction (P=0.010) for the subterranean clover yield. Flumetsulam treated 'Antas' had the highest subterranean clover yield of 2610 kg DM/ha or 92% of its total yield. Imazethapyr also increased the subterranean clover yield of 'Antas' to 2450 kg DM/ha compared with 1460 kg DM/ha in the control. The subterranean clover yield of 'Napier' also increased with both herbicide treatments (~2100 kg DM/ha) compared with the control (1390 kg DM/ha).

In comparison, the subterranean clover yield of 'Coolamon' was only increased by Flumetsulam. Flumetsulam 'Coolamon' had a subterranean clover yield increase of 760 kg DM/ha compared with the control. Imazethapyr had no effect on the subterranean clover yield for 'Coolamon'. Neither Imazethapyr nor Flumetsulam had an effect on the subterranean clover yields of the remaining cultivars ('Denmark', 'Monti', 'Narrikup' and 'Trikkala'). There was no effect of either herbicide (P=0.676) or cultivar (P=0.593) on the yield of white clover, which was low and averaged 110 kg DM/ha.

Imazethapyr and flumetsulam did reduce (P<0.001) the broadleaf weed yields by 560 and 510 kg DM/ha, respectively, compared with the control, with no difference in broadleaf weed yield between them. Grass weed yield was not affected (P=0.345) by either herbicide treatment compared with the control.

3.3.2.4 Harvest 2: 2 November 2018

The regrowth of plots at the second harvest showed 'Narrikup' (1860 kg DM/ha) and 'Napier' (1800 kg DM/ha) had the highest (P=0.016) total yield (Figure 3.7). In contrast, 'Antas' had the lowest total dry matter of 1360 kg DM/ha. The other four cultivars ranged from 1560-1690 kg DM/ha. Herbicide also had an effect (P<0.001) on total dry matter. Specifically, flumetsulam increased the total dry matter from 1470 kg DM/ha in the control to 1956 kg DM/ha. However, there was no difference in total dry matter between the imazethapyr (1520 kg DM/ha) treatment and the control (1470 kg DM/ha).

Subterranean clover yield was higher (P<0.001) in the flumetsulam (1500 kg DM/ha) than either the imazethapyr (1270 kg DM/ha) or control (960 kg DM/ha). Cultivar also had an effect (P=0.003) on subterranean clover yield. ‘Napier’ had the highest subterranean clover yield of 1520 kg DM/ha compared with ‘Antas’ which had the lowest (916 kg DM/ha).

White clover yield was lower (P=0.032) in imazethapyr (80 kg DM/ha) compared with the control and flumetsulam treatments which both had a white clover yield of ~145 kg DM/ha. Cultivar also had an effect (P=0.007) on white clover yield. ‘Monti’, ‘Antas’ and ‘Narrikup had higher yields (140-200 kg DM/ha) of white clover compared with ‘Napier’ and ‘Coolamon’ (~70 kg DM/ha).

Broadleaf weed yield was reduced (P<0.001) by over 50% by flumetsulam (120 kg DM/ha) and imazethapyr (100 kg DM/ha) compared with the control. The grass weeds increased (P<0.001) to 130 kg DM/ha in the flumetsulam treatment compared with 50 kg DM/ha in the control.

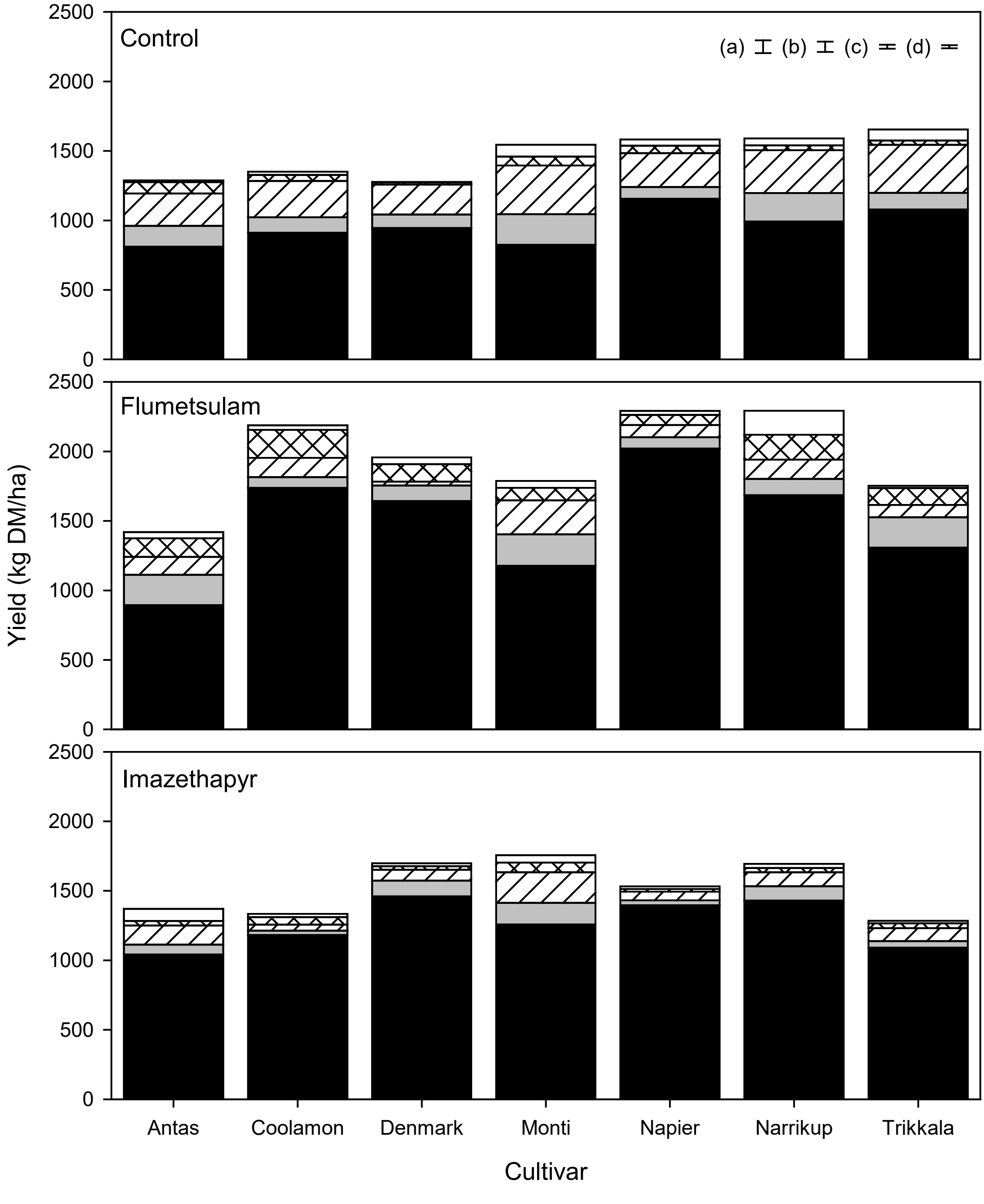

Figure 3.7 Dry matter (DM) yield of subterranean clover cultivars on 2 November 2018 after treatment with herbicides at establishment, at Iversen 9, Lincoln University, Canterbury, New Zealand. Categories are subterranean clover (■), white clover (■), broadleaf weed (▨), grass weed (⊠) and dead matter (□). Error bars are the SEM for (a)the main effect of cultivar for subterranean clover; (b) the main effect of herbicide for subterranean clover DM; (c) the main effect of herbicide for broadleaf weed DM, (d) the main effect of herbicide for grass weed DM.

3.3.2.5 Harvest 3: 6 December 2018

After the second defoliation, 'Antas' (4280 kg DM/ha) and 'Napier' (4100 kg DM/ha) produced highest (P=0.002) total dry matter yields (Figure 3.8). The cultivars with the lowest total dry matter yield were 'Coolamon' (2900 kg DM/ha) and 'Trikkala' (2940 kg DM/ha). Herbicide treatment had no effect on total dry matter yield.

At this third harvest, imazethapyr increased (P=0.026) the yield of subterranean clover by 500 kg DM/ha compared with the control (2050 kg DM/ha). Flumetsulam had no effect on the yield of subterranean clover. Cultivar also had an effect (P<0.001) on subterranean clover yield. 'Antas' (3280 kg DM/ha) and 'Napier' (3660 kg DM/ha) had the highest subterranean clover yields. The other five cultivars ranged from 1600-2600 kg DM/ha.

Cultivar had an effect (P=0.031) on the white clover yield. 'Monti', 'Denmark' and 'Trikkala' had the highest yield of white clover (~820 kg DM/ha) compared with 'Napier' which had the lowest (192 kg DM/ha).

There was a herbicide*cultivar interaction (P=0.015) for broadleaf weed yield. Flumetsulam and imazethapyr decreased the broadleaf weed yield in both 'Antas' and 'Coolamon'. Flumetsulam reduced the broadleaf weed yield by ~600 kg DM/ha in both 'Trikkala' and 'Monti'. Imazethapyr reduced the broadleaf weed yield from 800 kg DM/ha to 390 kg DM/ha in the 'Narrikup' treatment. Neither herbicide had any effect on the broadleaf weed yield in the 'Denmark' and 'Napier' treatments in the third harvest.

There was also a herbicide*cultivar interaction (P=0.017) for grass weed yield. Flumetsulam treated 'Monti' had the highest grass weed yield of 70 kg DM/ha compared with 3.4 kg DM/ha in the control. Imazethapyr also increased the amount of grass weed in 'Monti' to 36 kg DM/ha although not as much as flumetsulam. Flumetsulam (11 kg DM/ha) reduced the amount of grass weed in 'Trikkala' compared with the control (42 kg DM/ha). Neither herbicide treatment had an effect on grass weed yield for the remaining cultivars which averaged 11 kg DM/ha. In most cases the grass weed component was low and agronomically insignificant.

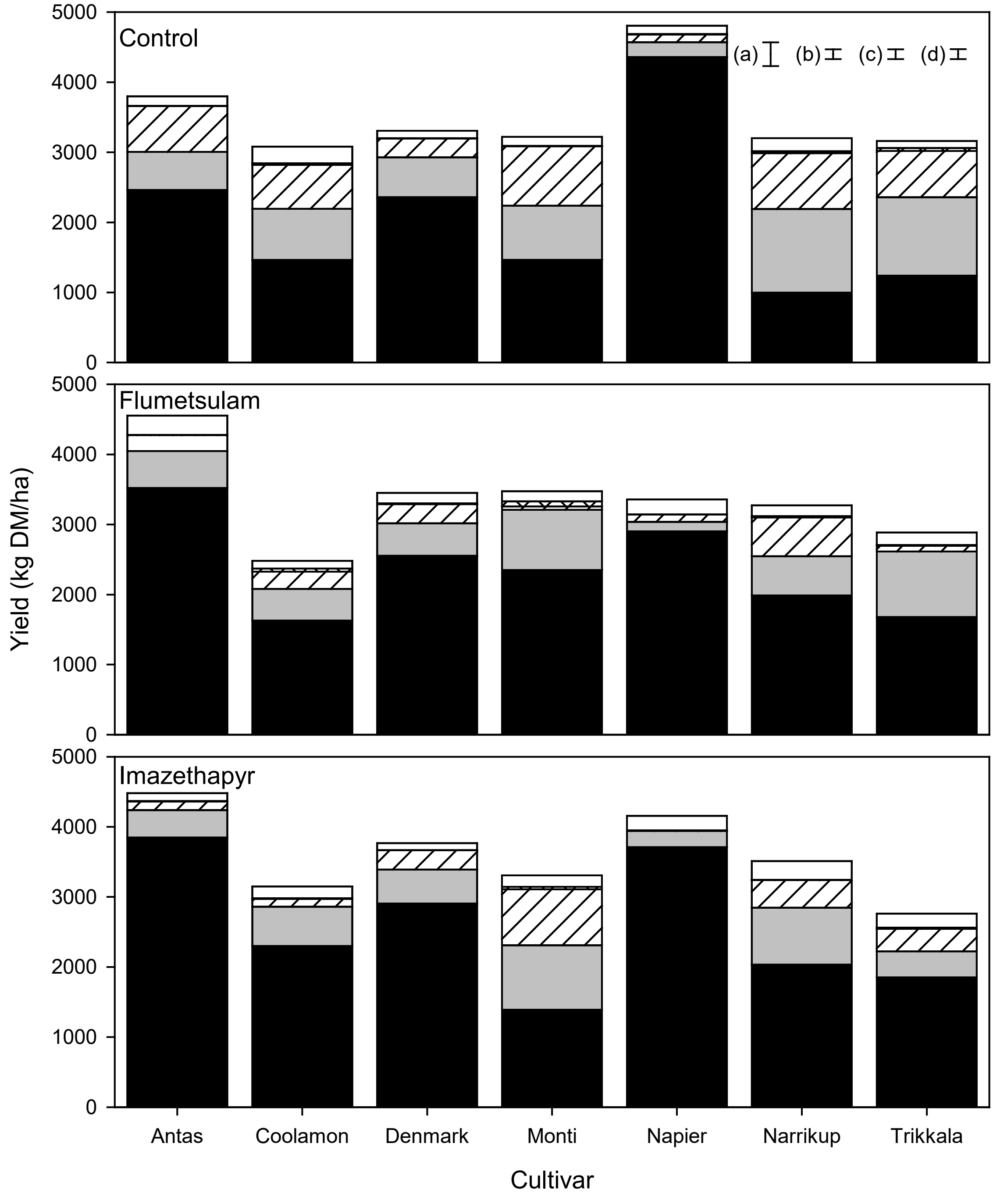

Figure 3.8 Dry matter (DM) of subterranean clover cultivars on 6 December 2018 after treatment with herbicides at establishment, at Iversen 9, Lincoln University, Canterbury, New Zealand. Categories are subterranean clover (■), white clover (■), broadleaf weed (▨), grass weed (⊠) and dead matter (□). Error bars are the SEM for (a) the main effect of cultivar for subterranean clover DM; (b) the main effect of herbicide for subterranean clover DM; (c) the main effect of cultivar for white clover DM; (d) the cultivar*herbicide interaction for broadleaf weed DM.

3.3.3 Botanical composition

The dry matter results presented in Section 3.3.2 were converted to percentages to normalise the botanical composition given differences in absolute yields among cultivars and herbicide treatments. Unless otherwise stated there were no interactions between herbicide and cultivar for the measured variables.

3.3.3.1 Harvest 1: 3 October 2018

The results of the botanical composition for the first harvest are shown in Figure 3.9. Flumetsulam (82.6%) and imazethapyr (84.4%) treatments had higher ($P<0.001$) percentages of subterranean clover than the control (60.5%). Cultivar also influenced ($P=0.032$) the percentage of subterranean clover. 'Antas' had a higher percentage of subterranean clover at 85.5% compared with 62.3% for 'Narrikup', which was the lowest.

The application of herbicide decreased ($P<0.001$) the percentage of broadleaf weed from 31.7% in the control to 6.63% in the imazethapyr treatment and 6.95% in the flumetsulam treatment. The herbicides had no effect ($P=0.466$) on the percentage of grass weeds.

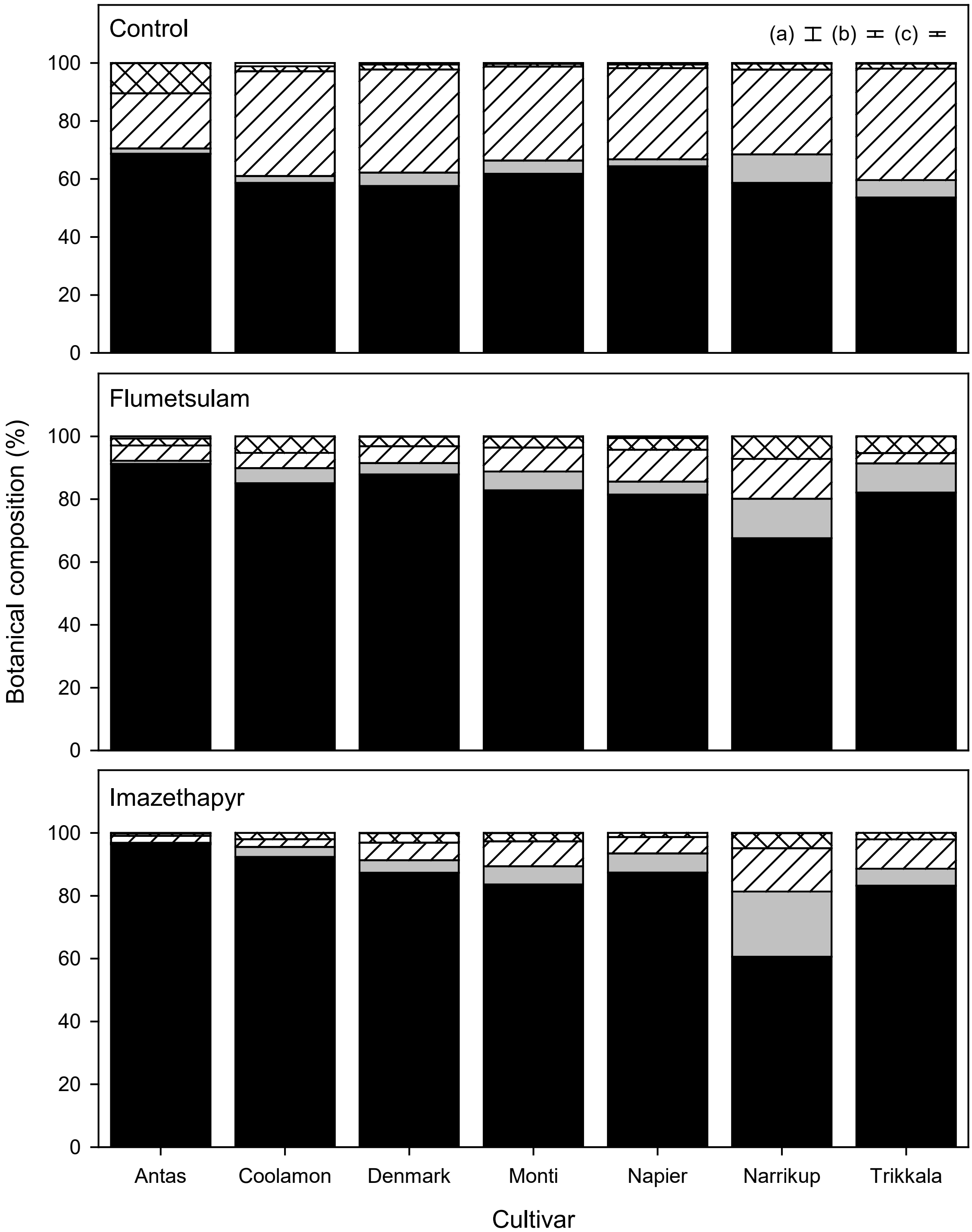

Figure 3.9 Botanical composition (%) of subterranean clover cultivars on 3 October 2018 after treatment of herbicides at Iversen 9, Lincoln University, Canterbury, New Zealand. Categories are subterranean clover (■), white clover (■), broadleaf weed (▨), grass weed (⊠) and dead matter (□). Error bars are the SEM for (a) the main effect of cultivar for subterranean clover %; (b) the main effect of herbicide for subterranean clover %; (c) the main effect of herbicide for broadleaf weed %.

3.3.3.2 Harvest 2: 2 November 2018

For the second harvest, imazethapyr increased (P=0.009) the percentage of subterranean clover more than flumetsulam (Figure 3.10). This was 83.3% in imazethapyr treated plots compared with 73.8% in the flumetsulam and 65.9% in the control plots. The cultivar with the highest percentage of subterranean clover was 'Napier' with 83.4%. 'Monti' had the lowest percentage at 62.9%.

Both imazethapyr and flumetsulam decreased the percentage of broadleaf weeds to ~6.9% when compared with the control (19.0%). The percentage of grass weeds increased (P=0.020) in the flumetsulam treated plots (7.0%) compared with the control (3.1%).

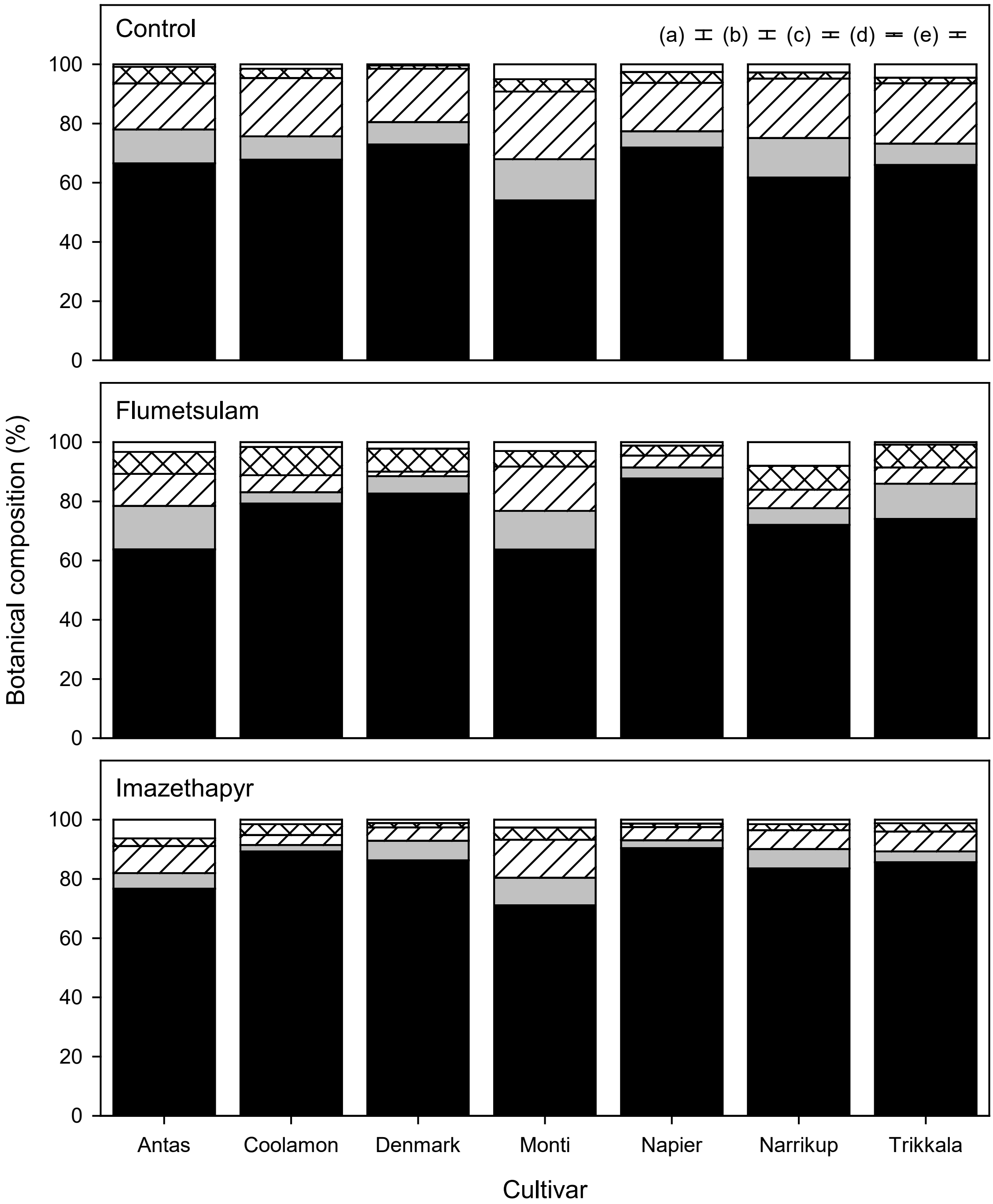

Figure 3.10 Botanical composition (%) of subterranean clover cultivars on 2 November 2018 after treatment of herbicides at Iversen 9, Lincoln University, Canterbury, New Zealand. Categories are subterranean clover (■), white clover (■), broadleaf weed (▨), grass weed (⊠) and dead matter (□). Error bars are the SEM for (a) the main effect of cultivar for subterranean clover %; (b) the main effect of herbicide for subterranean clover %; (c) the main effect of cultivar for white clover %; (d) the main effect of herbicide for white clover %; (e) the main effect of herbicide for broadleaf weed %.

3.3.3.3 Harvest 3: 6 December 2018

The results of the botanical composition for the final harvest are shown in Figure 3.11. There was an indication that the percentage of subterranean clover was increased ($P=0.052$) by the herbicide treatments from 56.3% in the control to ~70% for treated plots. 'Napier' was the cultivar with the highest ($P<0.001$) percentage of subterranean clover (88.4%). 'Antas' (74.9%), 'Denmark' (74.3%) and 'Coolamon' (62.4%) had the next highest percentage of subterranean clover. 'Trikkala' (55.7%), 'Monti' (52.1%) and 'Narrikup' (50.9%) had the lowest percentage of subterranean clover.

Cultivar also had an effect ($P=0.003$) on the percentage of white clover in the plots. 'Trikkala' (26.3%), 'Monti' (25.2%) and 'Narrikup' (25.4%) had the highest percentage of white clover. 'Napier' had the lowest percentage of white clover of 4.83%.

There was a herbicide*cultivar interaction for broadleaf weeds ($P<0.001$). Flumetsulam reduced the percentage of broadleaf weeds in 'Monti' to 1.20% compared with the control (26.9%) and imazethapyr (24.2%). Flumetsulam also reduced the percentage of broadleaf weeds in 'Trikkala' from 20.9% to 2.2%. There was no difference in broadleaf weed percentage between herbicide treatments and the control for the remaining cultivars.

The percentage of grass weeds was minimal at 0.3-2.2%.

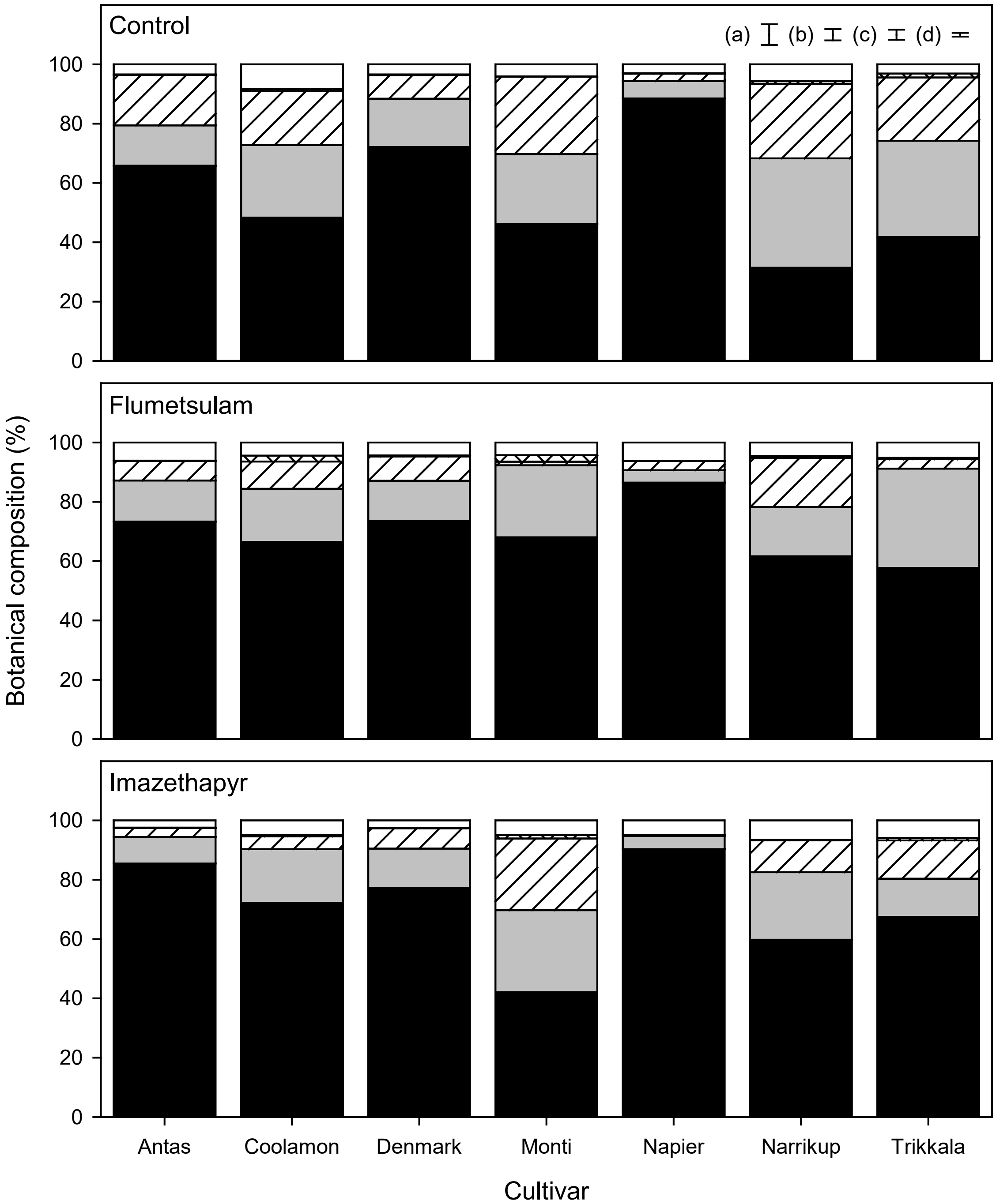

Figure 3.11 Botanical composition (%) of subterranean clover cultivars on 6 December 2018 after treatment of herbicides at establishment, at Iversen 9, Lincoln University, Canterbury, New Zealand. Categories are subterranean clover (■), white clover (■), broadleaf weed (▨), grass weed (⊠) and dead matter (□). Error bars are the SEM for (a) the main effect of cultivar for subterranean clover %; (b) the main effect of cultivar for white clover %; (c) cultivar*herbicide interaction for broadleaf weed %; (d) the main effect of cultivar for grass weeds %.

3.3.4 Broadleaf and grass weed control

At the final harvest, to determine the effect of the herbicide treatment on individual weed species, the broadleaf and grass weed components were sorted into species for the cultivar 'Monti'. Flumetsulam reduced (P=0.037) the dock yield from 290 kg DM/ha to 42 kg DM/ha (Figure 3.12). Imazethapyr treated 'Monti' had a dock yield of 196 kg DM/ha which did not differ from either the control or flumetsulam. The hedge mustard yield was reduced (P=0.013) by both herbicides from 177 kg DM/ha to 76 kg DM/ha for imazethapyr and 0 kg DM/ha for flumetsulam. Wire weed yield increased (P=0.012) in the imazethapyr treatment from 194 kg DM/ha to 456 kg DM/ha. Flumetsulam did not differ in wire weed yield compared with the control. For the remaining weed species there were no difference (P>0.233) in yields across all treatments, averaging 82 kg DM/ha for spurrey and 18 kg DM/ha for annual poa.

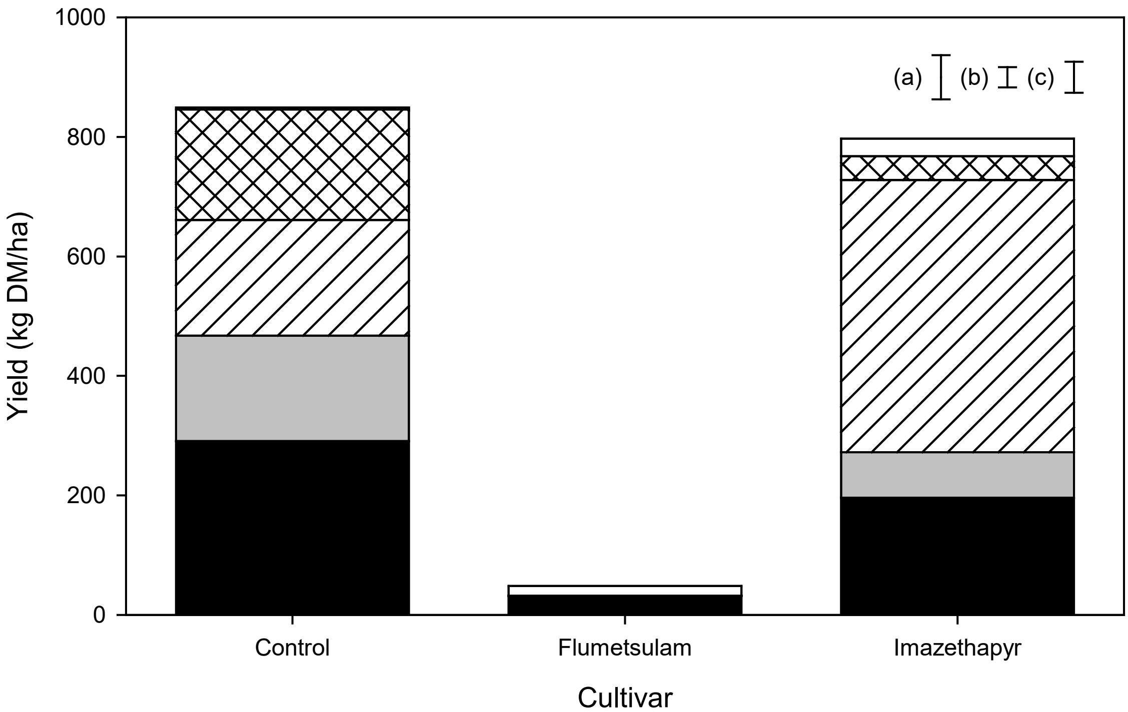

Figure 3.12 Broadleaf and grass weed dry matter (DM) yield from 'Monti' subterranean clover plots on 6 December 2018 after treatment with herbicides at establishment, at Iversen 9, Lincoln University, Canterbury, New Zealand. Categories are dock (■), hedge mustard (■), wire weed (▨), spurrey (⊠) and *poa annua* (□)**.** Error bars are the SEM for (a) the effect of herbicide for wire weed DM; (b) the effect of herbicide for hedge mustard DM; (c) the effect of herbicide dock DM.

3.3.5 Mean daily growth rates

Mean daily growth rate of the seven subterranean clover cultivars differed across all four periods (Figure 3.13). There were no cultivar*herbicide interactions for daily growth rate. Herbicide treatment also had no effect (P>0.067) on daily growth rates for any period. In the winter period (11/06-04/09/2018) 'Napier', 'Denmark' and 'Coolamon' had a higher (P=0.046) daily growth rate of 20-22 kg DM/ha/day than 'Narrikup', which averaged 13 kg DM/ha/day. In the early spring period (04/09-03/10/18), 'Antas' had the highest (P=0.019) daily growth rate of 105 kg DM/ha/day, which was not different from 'Coolamon' which averaged 88 kg DM/ha/day. There was no difference among the remaining cultivars, which ranged between 56 and 75 kg DM/ha/day.

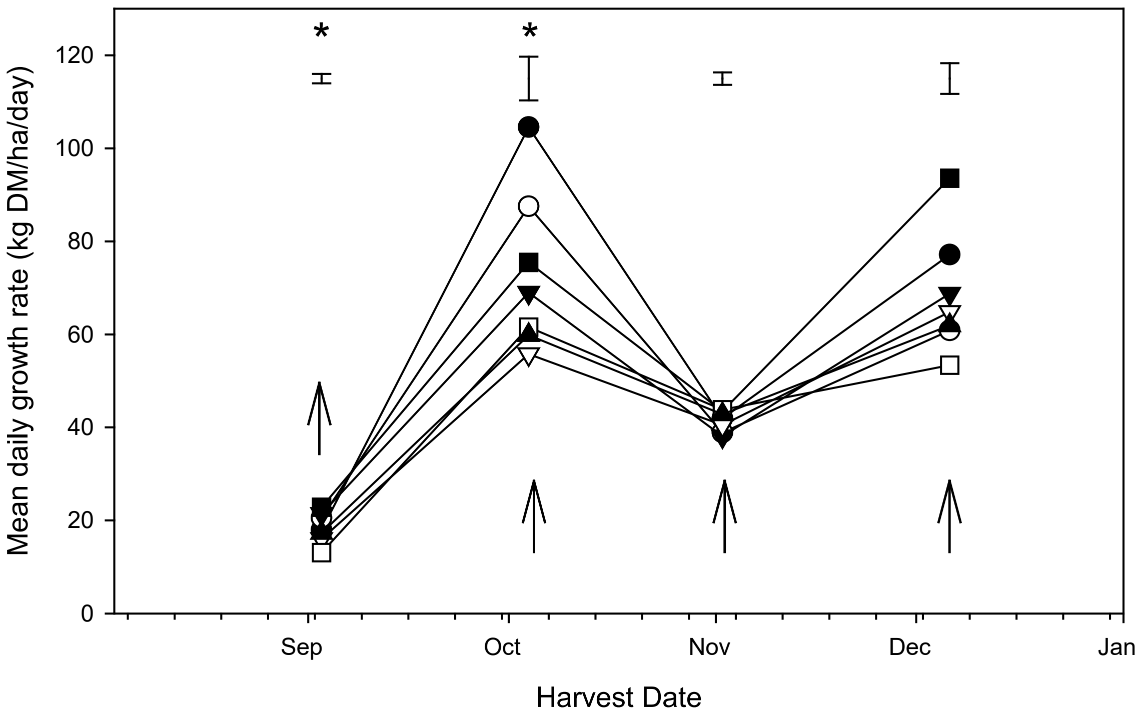

Figure 3.13 Mean daily growth rate of seven subterranean clover cultivars; Antas (●), 'Coolamon' (○), Denmark (▼), 'Monti' (▽), Napier (■), Narrikup (□), 'Trikkala' (▲), at Iversen 9, Lincoln University, Canterbury, New Zealand. Error bars are SEM for the main effect of cultivar. * = main effect of cultivar is significant (P<0.05). Arrows indicate harvests.

For the mid spring period (03/10-02/11/18) there was no difference ($P=0.959$) in daily growth rate among the cultivars, averaging 41 kg DM/ha/day. There was also no difference ($P=0.516$) in daily growth rate among the cultivars for the late spring period (02/11-06/12/18). The cultivars ranged from 61 kg DM/ha/day to 94 kg DM/ha/day.

3.3.6 Thermal time

The regression of subterranean clover DM yield against thermal time are shown Figure 3.14 with the slope and x intercepts of the cultivars in Table 3.9. A simple linear regression with groups was performed which had an R^2 value of 95.4% when T_b=0°C and 96.2% when T_b=3°C. 'Napier' had the highest ($P<0.001$) TAGR of 6.70 kg DM/ha/°Cd when T_b=0°C. 'Antas' and 'Denmark' had the next highest TAGR of 5.47 kg DM/ha/°Cd and 5.08 kg DM/ha/°Cd, respectively. The remaining four cultivars had an average TAGR of 3.72 kg DM/ha/°Cd, which was nearly half that of 'Napier'. The trend was similar when T_b=3°C but TAGRs were higher due to less thermal time being accumulated.

The x-axis intercept of the regression gives an indication of when linear growth begins. 'Monti' reached linear growth faster ($P=0.044$) at 888 °Cd, which occurred on 20 August 2018, than 'Napier' and 'Denmark' (Table 3.9). 'Denmark' and 'Napier reached linear growth at 1096 °Cd and 1022 °Cd respectively. This occurred 3-4 weeks later than 'Monti' on the 8 September 2018 for 'Denmark' and 15 September 2018 for 'Napier'. The remaining cultivars ranged between 913-1002 °Cd.

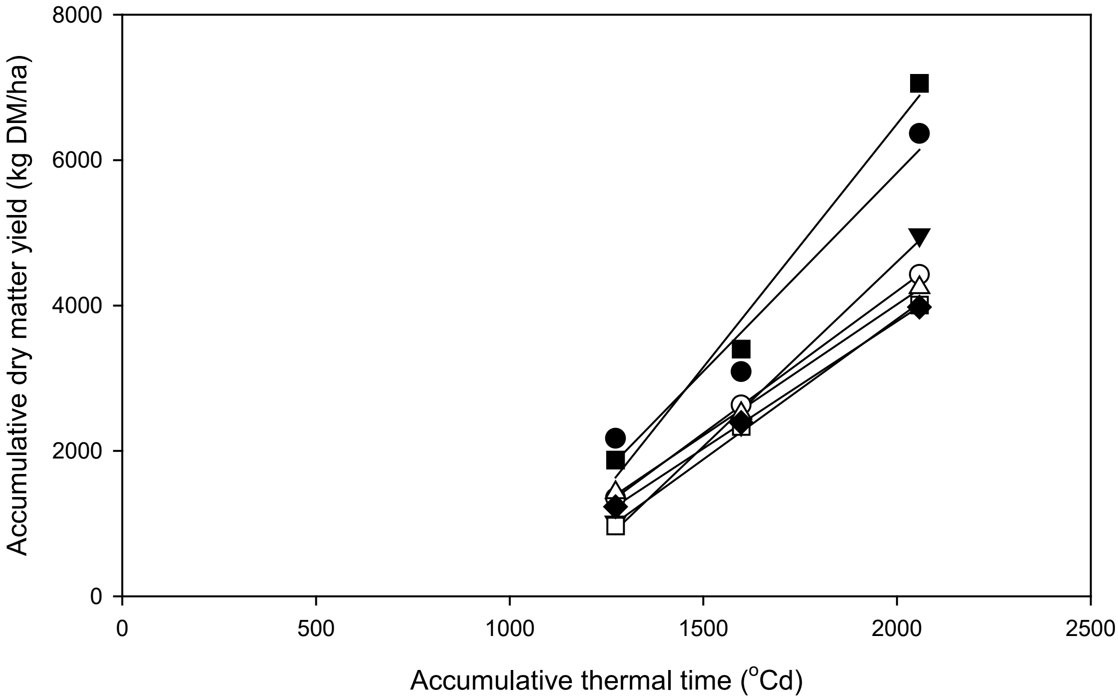

Figure 3.14 Subterranean clover dry matter yield (kg DM/ha) of seven subterranean clover cultivars; 'Antas' (●), 'Coolamon' (○), Denmark (▼), 'Monti' (▽), 'Napier' (■), 'Narrikup' (□), 'Trikkala' (▲), against accumulative thermal time (°Cd) with a base temperature of 0 at Iversen 9, Lincoln University, Canterbury, New Zealand.

Table 3.9 Slope and x-axis intercept of regression for subterranean dry matter yield against accumulative thermal time for seven cultivars at Iversen 9, Lincoln University, Canterbury, New Zealand.

Cultivar	T_b=0°C		T_b=3°C	
	Slope	X intercept	Slope	X intercept
Antas	5.47_{b}	936_{bc}	6.36_{b}	648_{bc}
Coolamon	3.92_{c}	921_{bc}	4.54_{c}	663_{bc}
Denmark	5.08_{b}	1096_{a}	5.90_{b}	785_{a}
Monti	3.61_{c}	888_{c}	4.19_{c}	605_{c}
Napier	6.70_{a}	1022_{ab}	7.79_{a}	721_{ab}
Narrikup	3.86_{c}	1002_{abc}	4.47_{c}	703_{abc}
Trikkala	3.50_{c}	913_{bc}	4.06_{c}	626_{bc}
P value	<0.001	0.044	<0.001	0.044
SEM	0.314	44.8	0.367	38.8

3.3.7 Canopy cover

There was a herbicide*cultivar interaction for canopy cover on two dates, 7 September and 16 October 2018, which are presented in Table 3.10. The mean canopy cover for each cultivar for the remaining dates is presented in Figure 3.15. On the 7 September 2018 there was a herbicide*cultivar interaction (P=0.032) for canopy cover (Table 3.10). There was no difference among the cultivars for the controls, with canopy cover ranging from 71-79%. Flumetsulam and imazethapyr reduced the ground cover in 'Denmark' from 74% in the control to ~60%. Only flumetsulam reduced the canopy cover in the remaining six cultivars.

Table 3.10 Canopy cover (%) of seven subterranean clover cultivars on 7 September and 16 October 2018 after treatment with herbicides, flumetsulam (FL) and imazethapyr (IM), at establishment, at Iversen 9, Lincoln University, Canterbury, New Zealand.

Cultivar	7 September 2018			16 October 2018		
	Control	FL	IM	Control	FL	IM
'Antas'	79.4_{a}	65.9_{bcde}	72.1_{abc}	44.9_{g}	38.7_{h}	38.1_{h}
'Coolamon'	77.7_{a}	62.9_{cde}	71.5_{abc}	55.6_{de}	59.6_{cde}	56.3_{cde}
'Denmark'	74.4_{ab}	59.2_{de}	58.5_{ef}	66.4_{b}	73.7_{a}	74.0_{a}
'Monti'	74.0_{ab}	58.0_{ef}	65.3_{bcde}	58.8_{cde}	61.8_{bcd}	60.9_{bcd}
'Napier'	75.1_{ab}	63.6_{cde}	67.6_{bcd}	55.2_{ef}	60.2_{bcd}	53.9_{f}
'Narrikup'	70.9_{bc}	53.3_{f}	66.5_{bcd}	59.2_{cde}	62.1_{bcd}	62.2_{bc}
'Trikkala'	75.0_{ab}	57.8_{ef}	65.6_{bcde}	57.1_{cde}	62.0_{bcd}	56.0_{cde}
P value – HB*CV		0.032			0.018	
SEM		3.496			2.266	

For the next measurement, on 19 September 2018, 'Antas', 'Coolamon' and 'Napier' had higher (P=0.014) canopy convers of ~85% but 'Narrikup' and 'Denmark' averaged 78% (Figure 3.15). Herbicide also had an effect (p=0.012) on canopy cover at this date with flumetsulam having a lower canopy cover of 76% compared with imazethapyr (83%) and the control (87%). There was no difference among cultivars (P=0.568) or herbicide treatments (P=0.167) on 3 October 2018 with canopy cover averaging 95%, suggesting that canopy closure had been achieved (Section 3.2.7.4).

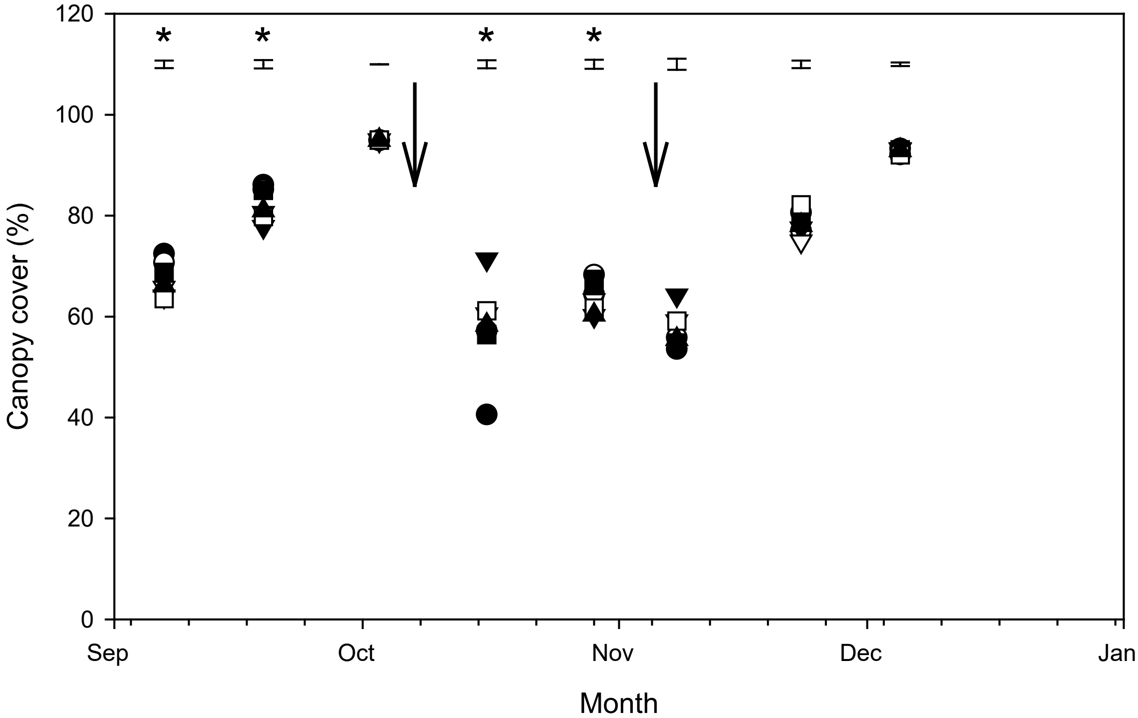

Figure 3.15 Mean canopy cover (%) of seven subterranean clover cultivars; Antas (●), 'Coolamon' (○), Denmark (△), 'Monti' (▽), Napier (■), Narrikup (□), 'Trikkala' (▲) from 7 September 2018 – 6 December 2018 at Iversen 9, Lincoln University, Canterbury, New Zealand. Error bars represent the SEM for the main effect of cultivar. * = main effect of cultivar is significant (P<0.05). Arrows show when Experiment 1 was grazed.

There was a herbicide*cultivar interaction (P=0.018) again on the 16 October 2018, after the first grazing (Table 3.10). 'Antas' had the lowest canopy cover at this time with 45% for the control which was further reduced in the flumetsulam and imazethapyr treatments to ~38%. Flumetsulam and imazethapyr treated 'Denmark' had the highest canopy cover of 74% which was increased from the control of 66%. In contrast, canopy cover was only increased by flumetsulam for 'Napier' from 55% to 60%. The remaining cultivars did not differ in canopy cover between the herbicide and control treatments, ranging between 55-62%.

On the 29 October 2018 there was a cultivar effect (P=0.028) on canopy cover. 'Napier' and 'Coolamon' had ~68% canopy cover which was higher than 'Narrikup' (62%), 'Trikkala'

(60%) and 'Denmark' (60%) (Figure 3.15). Canopy cover was reduced (P=0.007) in the flumetsulam treated plots to 56% compared with the control (71%). The imazethapyr treatment averaged 64% canopy cover which did not differ from either the control or flumetsulam.

There was no difference in canopy cover among cultivars and treatments for the remaining measurements. Canopy cover averaged 57% on the 8 November 2018, 78% on the 23 November 2018 and 93% on the 5 December 2018.

3.3.8 Phytotoxicity assessment

The mean EWRS scores for all seven cultivars are presented in Figure 3.16. The two herbicide treatments had higher EWRS scores than the control across all dates but never differed from each other. At the first measurement, 7 DAT, imazethapyr and flumetsulam had a EWRS score of 2.4 (P<0.001). This increased to ~3.5 16 DAT and then to ~4.0 30 DAT. At 51 DAT, the EWRS scores decreased to 3.4, then increased again to 4.3 at the final measurement date (84 DAT).

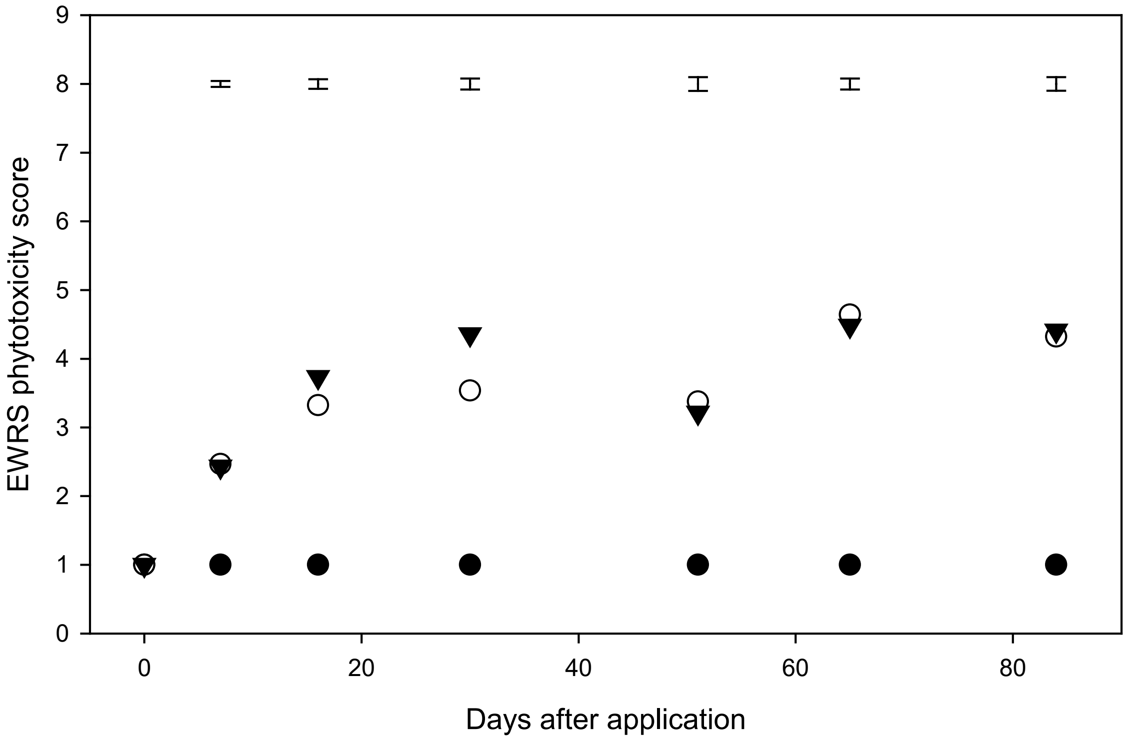

Figure 3.16 Mean EWRS phytotoxicity score across all seven cultivars for three herbicide treatments from 4 July – 26 September 2018 at Iversen 9, Lincoln University, Canterbury, New Zealand. Categories are control (●), flumetsulam (○), imazethapyr (▼). Error bars are the SEM.

Cultivar had an effect on EWRS score for two dates, 7 and 84 DAT (Table 3.11). ‘Antas’ and ‘Monti’ had higher (P=0.043) EWRS scores of 2.04 and 2.33 respectively, compared with ‘Denmark’ (1.63) and ‘Trikkala’ (1.79) at 7 DAT. The remaining cultivars ranged from 1.96-2.00. 84 DAT ‘Coolamon’ had a higher (P=0.029) EWRS score of 3.79 compared with ‘Antas’ (2.92), ‘Denmark’ (2.92) and ‘Napier’ (2.96).

Table 3.11 Mean EWRS phytotoxicity scores for all herbicide treatments for seven subterranean clover cultivars 7 DAT and 84 DAT at Iversen 9, Lincoln University, Canterbury, New Zealand.

Cultivar	7 DAT 11 July 2018	84 DAT 26 September 2018
'Antas'	2.04 a	2.92 c
'Coolamon'	1.96 ab	3.79 a
'Denmark'	1.63 b	2.92 c
'Monti'	2.33 a	3.42 ab
'Napier'	2.00 ab	2.96 bc
'Narrikup'	2.00 ab	3.21 abc
'Trikkala'	1.79 b	3.50 ab
P value	0.043	0.029
SEM	0.0716	0.1449

3.3.9 Re-emergence: Autumn 2019

At the first measurement on 21 February 2019, there was no difference (P=0.398) in the number of seedlings among the cultivars, which ranged between 570-1100 seedlings/m^2 (Table 3.12). On 15 March 2019, 'Monti' and 'Napier' had higher (P<0.001) emergence scores, 6.00 and 5.75 respectively, than 'Antas', 'Coolamon' and 'Narrikup'. 'Coolamon' and 'Narrikup' had scores of 5.00 and 4.75 and 'Antas' had the lowest score of 1.75.

Table 3.12 Seedling/m^2 and emergence score for subterranean clover seedlings on 21 February 2019 and 15 March 2019 at Iversen 9, Lincoln University, Canterbury, New Zealand.

Cultivar	Seedlings/m^2 21/02/2019	Emergence score* 15/03/2019
'Antas'	570	1.75d
'Coolamon'	1100	5.00bc
'Denmark'	730	5.50ab
'Monti'	860	6.00a
'Napier'	910	5.75a
'Narrikup'	930	4.75c
'Trikkala'	600	5.50ab
P value	0.398	<0.001
SEM	66.8	0.2689

*Note; Emergence scores were equated to population counts by Teixeira *et al.* (2018). See Table 3.8.

3.3.10 Residual dock control

The results of the residual dock control by the herbicides the following autumn are shown in Table 3.13. Imazethapyr reduced (P<0.001) the amount of docks from a score of 4.3 in the control to 2.1 (Plate 3.1). There was no difference between flumetsulam and the control.

Table 3.13 Score of dock control eight months after application, 15 March 2019, of herbicides at Iversen 9, Lincoln University, Canterbury, New Zealand.

Treatment	Score
Control	4.3_a
Flumetsulam	4.0_a
Imazethapyr	2.1_b
P value	<0.001
SEM	0.2552

Plate 3.1 Residual control of docks by imazethapyr on 15 March 2019. Red lines indicate strip plot boundaries with imazethapyr in the centre and control plots directly either side.

3.4 Discussion

3.4.1 Seedling establishment

High populations of subterranean clover were established evenly across all plots allowing herbicide effects on cultivars to be assessed. ‘Antas’, ‘Napier’ and ‘Monti’ had the highest emergence rates of >84% (Table 3.1). Subterranean clover seedlings began to emerge on 1 May 2018. Seedling population peaked on 21 June 2018 with seedling populations ranging between 210-400±13.5 plants/m^2. This indicated >55% field emergence rate for all cultivars

(Table 3.1). After this the seedling population decreased which was likely due to intraspecific competition. There was a slight increase in seedling population indicated from the 4 September 2018, due to plants being dug up and counted, which made the measurement more accurate. The population stabilised at; ~240±16.8 plants/m^2 for 'Denmark' and 'Coolamon', ~175±16.8 plants/m^2 for 'Napier', 'Trikkala' and 'Narrikup', and ~110±16.8 plants/m^2 for 'Antas' and 'Monti'. 'Denmark' and 'Coolamon' both have small seed weights so more seed would have been sown than for the large seeded cultivars 'Antas' and 'Monti' (Table 3.1). 'Antas' is also a large leafed plant, compared with the small leafed Denmark, so the competition between plants would have been larger, which could have resulted in greater self-thinning.

No reduction in subterranean clover population was seen after herbicides were applied on the 4 July 2018 which suggests neither herbicide affected subterranean clover populations when applied at the 4-5 leaf growth stage.

3.4.2 Subterranean clover yields

3.4.2.1 4 September 2018

'Coolamon', 'Denmark', and 'Napier' had the highest subterranean clover yields on 4 September 2018 of ~1800±175 kg DM/ha (Figure 3.4). When canopy cover was measured three days later 'Coolamon' and 'Napier' had the highest canopy covers of ~70±3.5% which suggests they were intercepting more light which would explain the higher yields. 'Denmark' had the lowest canopy cover of 64±3.50% but is short and had highest number of seedlings which may account for the high yield. 'Narrikup' had the lowest subterranean clover yield of 1110±175kg DM/ha from a similar canopy cover (64±3.50%) to 'Denmark' but its more upright structure may mean there was less leaf area present to intercept light.

There was no effect of herbicide on subterranean clover yield at this harvest. Weeds were not large enough at this point to create significant competition so there was no increase in subterranean clover yield in the herbicide treatments from elimination of broadleaf weeds.

The subterranean clover yield for early spring in this experiment is low. March sowing is recommended for subterranean clover in Canterbury (Moot *et al.*, 2003). In an experiment also conducted in Iversen Field at Lincoln University, subterranean clover which germinated in March yielded around 7000 kg DM/ha by mid-September or 5200 kg DM/ha more than the highest yielding cultivars in this experiment. In the previous experiment, subterranean clover that germinated in May, from a 7 May sowing date, yielded 1800 kg DM/ha in mid-September which is comparable with the yields produced in Experiment 1. In both cases, canopy closure was not achieved before growth slowed in late autumn and winter which would have reduced the amount of light interception and therefore growth rates.

3.4.2.2 Highest yielding cultivars: Antas and Napier

'Antas' and 'Napier' were the highest yielding cultivars with ~8200±398 kg DM/ha of total dry matter for the season (Figure 3.5). There was no difference in total yield between the herbicide treatments and the control but subterranean yield increased in the imazethapyr and flumetsulam treatments by ~1200±173 kg DM/ha. This was due to the reduction in broadleaf weeds which probably allowed the subterranean clover to capture more light. 'Antas' and 'Napier' had an increase of subterranean clover yield of ~2200±329 kg DM/ha compared with the next highest yielding cultivars, suggesting that 'Antas' and 'Napier' most suited to take advantage of the reduction in weed competition.

At the first harvest on 3 October 2018, the subterranean clover yield was increased by imazethapyr and flumetsulam by 42% for 'Antas' and 35% for 'Napier' (Figure 3.6). Imazethapyr and flumetsulam did not negatively affect the growth of these cultivars. 'Antas' had the highest growth rate at this early spring time period of 104±9.42 kg DM/ha while 'Napier' had one of the highest late winter growth rates (Figure 3.13). This allowed these cultivars to take advantage of the increased resources available to them once the weeds were controlled.

However, 'Antas' had one of the lowest subterranean clover yield of 910±181 kg DM/ha at the second harvest on 2 November 2018. After the first grazing, 'Antas' had the lowest canopy cover of 40±1.6%, compared with ~60±1.6% for the other cultivars (Figure 3.15). 'Antas' is a large leafed cultivar with an upright growth habit compared with cultivars such as 'Denmark' that have a prostate growth habit, close to the ground. This appears to have made ‘Antas’ susceptible to being overgrazed with the most leaf material being grazed, leaving only petioles behind. This lower canopy cover reduced the ability of ‘Antas’ to intercept light resulting in its low clover yield. ‘Napier’ is also a large leafed cultivar and had one of the highest subterranean clover yields of 1500±181 kg DM/ha across the three treatments, which is consistent with its canopy cover of 50±1.6%. Subterranean clover yields were higher in the flumetsulam treatment than the imazethapyr treatment and the control for all seven cultivars, even though both herbicide treatments removed around 270±27 kg DM/ha of broadleaf weeds. This suggests that imazethapyr had some impact on subterranean clover growth at this time, 17 weeks after application. This could be due to imazethapyr having longer soil residual activity than flumetsulam (Hollaway *et al.*, 2006b).

‘Antas’ had recovered by the third harvest, where it had the highest subterranean clover yield, along with ‘Napier’ of ~3300±241 kg DM/ha. The grazing after the second harvest was shorter, three days compared with five days the previous grazing, and the canopy cover of ‘Antas’ did not differ from the other cultivars after grazing. November was wetter than average (Figure 3.1) and ‘Antas’ and ‘Napier’ were able to take advantage of this longer than normal growing season. This may partly be because they are late flowering cultivars (Table 2.1) and remained in vegetative growth for longer. In contrast to the previous harvest, both imazethapyr and flumetsulam increased the subterranean yield compared with the control. Any impact of the herbicides on subterranean growth appeared to have been overcome by the advantage of broadleaf weed control.

3.4.2.3 Subspecies subterraneum cultivars

The three ssp. *subterraneum* cultivars, ‘Denmark’, ‘Coolamon’ and ‘Narrikup’ did not differ in total subterranean clover yield for the season, averaging ~4500±329 kg DM/ha or 54% of the highest yielding cultivars, ‘Antas’ and ‘Napier’ (Figure 3.5). At the first harvest, 3 October 2018, the subterranean clover yield of flumetsulam and imazethapyr ‘Denmark’ and ‘Narrikup’ did not differ from the control. Both herbicide treatments removed ~530±55.0 kg DM/ha of broadleaf weeds. It was expected that subterranean clover growth would increase when competition was reduced unless the herbicide inhibited growth. This suggests that both herbicides had some impact on the growth of these cultivars as they did not take advantage of the reduced broadleaf weed competition compared with ‘Antas’ and

‘Napier’. In contrast, the subterranean clover yield of ‘Coolamon’ was increased by 42% when flumetsulam was applied. The imazethapyr treated ‘Coolamon’ did not differ from the control. Both herbicide treatments reduced broadleaf weeds by the same amount. This suggests only imazethapyr affected the growth of ‘Coolamon’, as growth increased in the flumetsulam treatment. Therefore, it may be prudent to recommend flumetsulam ahead of imazethapyr for ‘Coolamon’.

At the second harvest, 2 November 2018, all three ssp. *subterraneum* cultivars had higher subterranean clover yields than ‘Antas’ and ‘Monti’ averaging 1330±92.0 kg DM/ha. ‘Denmark’ had the highest canopy cover of 71±1.63% after grazing on 16 October 2018 due to its low growth form which allowed the plant to persist under intensive grazing. However, by the 29 October 2018 ‘Coolamon’ had 68% canopy cover which was higher than ‘Denmark’ (60%) and ‘Monti’ (63%). This means all three cultivars likely intercepted a similar amount of light between the first and second harvest resulting in similar clover yields. The canopy cover of ‘Denmark’ decreased between these two measurements, from 71% to 60%, which was unexpected as there was no grazing during this time period. One possibility is sampling in higher growth areas in the first harvest and then sampling low growth areas for the next measurement.

At the final harvest on 6 December 2018, ‘Coolamon’ and ‘Narrikup’ had lower subterranean clover yields of ~1700±241 kg DM/ha than ‘Denmark’. ‘Denmark’ had a subterranean clover yield of 2600±241 kg DM/ha, which did not differ from ‘Antas’. As with ‘Antas’ and ‘Napier’, ‘Denmark’ is a late flowering cultivar which meant it was able to take advantage of the November rain and grow more than the mid-flowering cultivars ‘Coolamon’ and ‘Narrikup’ (Nichols *et al*., 2013a).

3.4.2.4 Subspecies yanninicum cultivars

‘Napier’ yielded more subterranean clover for the season than the other ssp. *yanninicum* cultivars, ‘Monti’ and ‘Trikkala’ as previously discussed in Section 3.4.2.2. ‘Monti’ and ‘Trikkala’ had a total subterranean clover yield of 4250±329 kg DM/ha and 3980±329 kg DM/ha, respectively (Figure 3.5). This did not differ from the subterranean clover yields of the ssp. *subterraneum* cultivars, with the exception of ‘Trikkala’ that was lower than the

'Denmark' yield. Both herbicides increased the total subterranean clover yield for the season for all three cultivars. Previous research has shown ssp. *yanninicum* to yield poorly and have a low herbicide tolerance in a low rainfall year (Lewis, 2017). The higher rainfall of this experiment led to increased growth of the ssp. *yanninicum* which may have resulted in the faster metabolism of the herbicides (Cobb and Reade, 2010).

For the first harvest, the flumetsulam and imazethapyr treatments did not differ from the controls from 'Monti' and 'Trikkala', suggesting that growth was limited by the herbicides. However by the second harvest, the flumetsulam treatment increased growth compared with the control and imazethapyr. 'Monti' averaged 1090±92.0 kg DM/ha of subterranean clover across all herbicide treatments, which was lower than 'Narrikup'. 'Trikkala' averaged 1160±92.0 kg DM/ha of subterranean clover which was the same as the ssp. *subterraneum* cultivars.

Ssp. *yanninicum* is adapted to higher rainfall areas than ssp. *subterraneum* (Katznelson, 1970). With the high rainfall in spring, 'Napier' outperformed the ssp. *subterranean* cultivars but 'Monti' and 'Trikkala' did not. At the third harvest, 'Monti' and 'Trikkala' had low subterranean clover yields of 1700±241 kg DM/ha and 1590±241 kg DM/ha, respectively. These were lower than the subterranean clover yields for 'Antas', 'Napier' and 'Denmark'. This suggests that they are lower yielding than 'Napier' or are not as suited to the conditions in this experiment. 'Monti' and 'Trikkala' are early flowering cultivars (Table 2.1) which means they have a shorter growing season and vegetative growth had likely naturally slowed by this point.

3.4.3 Thermal time

The TAGR of the subterranean clover cultivars ranged from 3.50-6.70 kg DM/ha/°Cd when T_b=0°C (Section 3.3.6). Tonmukayakul (2009) reported the TAGR for cocksfoot (*Dactylis glomerata* L.)/'Denmark' subterranean clover pastures to be 5.9 kg DM/ha/°Cd during spring with a Tb=0°C. This is slightly higher than 'Denmark' and 'Antas' that had a TAGR of 5.08±0.314 kg DM/ha/°Cd and 5.47±0.314 kg DM/ha/°Cd, respectively.

Napier had the highest TAGR of 7.8±0.367 kg DM/ha/°Cd when Tb=3°C which was similar to the 8.3 kg DM/ha/°Cd reported for cocksfoot/subterranean clover pasture in spring

(Mills *et al.*, 2008). The four cultivars with the lowest TAGR which averaged 4.315±0.367 kg DM/ha/°Cd was slightly higher than the 3.2 kg DM/ha/°Cd reported for unfertilised 'Wana' cocksfoot where T_b=3°C (Mills, 2007). This is expected as subterranean clover can fix its own nitrogen.

All cultivars had a lag phase where growth was not linearly related to temperature as the x-axis intercept of the regression did not equal 0. This is likely due to low cover over winter leading to slow growth.

3.4.4 White clover yield

White clover yields were low at the first harvest, around 6% of the total yield, and did not differ among the cultivars or herbicide treatments (Figure 3.6). At the second harvest, after the first grazing, the yield of white clover, ~160±22.0 kg DM/ha, was higher in the 'Monti', 'Antas' and 'Narrikup' plots (Figure 3.7). White clover is susceptible to shading and stolon growth decreases under reduced light intensity (Caradus and Chapman, 1991). 'Antas' had been grazed the hardest and had 40±1.63% canopy cover after grazing on 16 October 2018, which would allow more light into the lower canopy and onto germinating white clover seedlings. In comparison, 'Denmark' had the highest canopy cover after grazing of 71±1.63% and one of the lowest white clover yields at the second harvest of 110±22.0 kg DM/ha. This suggests an antagonism between subterranean and white clover.

At the third harvest on 6 December 2018, white clover growth had increased from the previous harvest to 840±141 kg DM/ha in 'Monti', 'Narrikup' and 'Trikkala' (Figure 3.8) which had higher yields of white clover than 'Napier' (190±141 kg DM/ha). Rainfall in November 2018 was over double the long term mean. White clover requires a high rainfall (Knowles *et al.*, 2003; Monk *et al.*, 2016) to sustain growth from shallow roots and so there was an increase in white clover growth during November compared with October. 'Napier' plots always had a high proportion of subterranean clover in the previous harvests which would prevent white clover growth. 'Monti', 'Narrikup' and 'Trikkala' were some of the lowest yielding subterranean clover cultivars and were therefore not as competitive and likely had more white clover seedlings present. This would explain the higher yields of white clover once rainfall occurred.

3.4.5 Broadleaf weed control

Both flumetsulam and imazethapyr gave effective broadleaf weed control with a reduction of about 1000±140 kg DM/ha of broadleaf weeds for the season. Broadleaf weed yield was reduced by 530±55.0 kg DM/ha in the first harvest and 170±27.1 kg DM/ha in the second harvest. There was a cultivar*herbicide interaction at the third harvest (Figure 3.7). Imazethapyr and flumetsulam did not reduce the broadleaf weeds for 'Denmark' and 'Napier'. This is likely due to both controls for these cultivars having a low amount broadleaf weeds, 110±37.2kg DM/ha for 'Napier' and 260±37.2 kg DM/ha for 'Denmark', compared with other cultivars controls, e.g. 850±37.2 kg DM/ha for 'Monti'. 'Denmark' has a low growth habit which appeared to shade broadleaf weed seedlings and stop them from establishing. This is also shown by the canopy cover for 'Denmark' being high (Figure 3.15), especially after grazing when other cultivars had more bare ground, which allowed weeds in. 'Napier' always had high subterranean clover yields throughout the experiment which likely outcompeted the weeds in the control. 'Antas' was also high yielding but the first grazing left a lot of bare ground for weeds to exploit. Flumetsulam-treated 'Monti' and 'Trikkala' had reduced broadleaf weeds compared with imazethapyr. It is unclear why this would be the case for these two cultivars when imazethapyr and flumetsulam equally reduced broadleaf weeds in the remaining cultivars.

At the final harvest, the broadleaf weed component was separated into different weed species for 'Monti' (Figure 3.12). Flumetsulam reduced the yield of dock and hedge mustard. Imazethapyr also reduced the yield of hedge mustard but did not control wire weed, with yields increasing compared with the control. The control of hedge mustard may have allowed for more wire weed to grow. The yield of spurrey did not differ between the herbicide treatment and control but was low at 82 kg DM/ha. This indicates flumetsulam had greater control of broadleaf weeds than imazethapyr, which is not the case when total seasonal yields are looked at. Broadleaf weed species were only separated for one cultivar 'Monti' which behaved differently from the other cultivars. To get a better understanding of which broadleaf weeds are controlled by the herbicides separations need to be done on multiple cultivars.

3.4.6 Grass weed control

Grass weeds, predominately *Poa annua*, made up a small component of total yield of <150±41.8 kg DM/ha for the season with there being no difference between the treatments and the control (Figure 3.5). For the second harvest, after the subterranean clover had been grazed hard, grass weeds doubled in the flumetsulam treatment compared with the control and imazethapyr (Figure 3.7). This was likely due to the control of broadleaf weeds opening space in the pasture and allowing annual grass seedlings to germinate and grow and the subterranean clover was recovering from being grazed and therefore not competitive. In the control treatment the grass weeds appear to be outcompeted by the larger broadleaf weeds.

At the third harvest, the grass weed yield in 'Monti' increased due to flumetsulam and imazethapyr, although not as greatly for the latter, compared with the control (Figure 3.8). 'Monti' had one of the lowest yields of subterranean clover. This seems to have allowed more grass weeds to grow than in other higher yielding cultivars. In contrast, grass weed yield decreased in the flumetsulam treated 'Trikkala' plots, although not compared with the imazethapyr treatment. This was unexpected as flumetsulam has not been reported to control grass weeds (Lonza, 2018) but it could be due to the higher subterranean clover yields in the flumetsulam plot compared with the control. The remaining five cultivars had no difference in grass weed yield across the treatments.

Spinnaker®, the imazethapyr herbicide used in this experiment, claims to control *Poa annua* (BASF, 2016) and imazethapyr has been shown to control other annual grass weeds (Gimenez *et al.*, 1998). Flumetsulam and imazethapyr have different chemical structures (Figure 2.1) which means they would have different species selectivity (Ladner, 1990). It seems that imazethapyr does have some control of *Poa annua*, with there being no increase in grass weeds for the second harvest. Imazethapyr also had a lower increase in grass weed compared with flumetsulam for 'Monti' in the third harvest. The levels of grass weeds in this experiment were agronomically insignificant and more of a difference may have been seen between the two treatments if more grass weeds had been present at the experimental site. As imazethapyr and flumetsulam performed similarly in other areas, such as phytotoxicity and subterranean clover yield, it may be advantageous to use imazethapyr in subterranean clover for its potential to control some annual grass weeds.

3.4.7 Phytotoxicity

Both herbicides had slight phytotoxic effects, with an EWRS phytotoxicity score of 2.5±0.08 7 DAT. This then increased and stabilised at an EWRS score of ~3-4 for the remaining assessments (Figure 3.16). At 84 DAT both herbicide treatments had an EWRS score of 4.3±0.20, which corresponds to some yellowing of leaves but no visible reduction in yield. This is consistent with dry matter measurements taken at the same time which showed herbicide had no effect on subterranean clover yield (Figure 3.4). These results are consistent with Lewis (2017), who showed subterranean clover sprayed with the same rates of imazethapyr and flumetsulam at a similar growth stage had EWRS scores ranging from 2.9-3.2 for 7 to 63 DAT. EWRS scores in this experiment are slightly higher which is not unexpected as herbicide effects can differ depending on environmental conditions such as rainfall and temperature (Dear *et al.*, 1995) and due to different individuals assessing the phytotoxic effects.

'Antas' and 'Monti' had higher EWRS scores of ~2±0.1 compared with 'Denmark' and 'Trikkala' 7 DAT (Table 3.11). Lewis (2017) found a correlation between EWRS score and plant pubescence, with hairy plants showing the least phytotoxic damage. This does not seem to be the case in this experiment as 'Antas', 'Denmark', 'Monti' and 'Trikkala' were all found to have a low pubescence score by Lewis (2017) but had different EWRS scores in this experiment. Higher phytotoxic damage may be instead due to the larger leaved cultivars like 'Antas' absorbing more herbicide than the small leaved cultivars, 'Denmark' and 'Trikkala'. However, the difference between EWRS scores is small and was not present at the next measurement date 16 DAT so was inconsequential and did not affect subsequent growth.

3.4.8 Re-emergence: Autumn 2019

Subterranean clover re-emergence the following autumn was measured on 21 February 2019. The re-emergence seedling populations were higher than the original emergence in 2018. There was no difference in emergence among the cultivars with seedling populations ranging from 570-1100±66.8 plants/m^2 (Table 3.12). There was 51 mm of rainfall from 1

January – 21 February 2019, with 21.2 mm of rain on 14 January, which was sufficient rain for germination to occur (Teixeira *et al.*, 2018). Subterranean clover is prone to 'false strikes' where summer rainfall triggers a germination event that is likely to fail to establish due to insufficient rainfall after germination. As this February germination was likely a 'false strike' the plots were sprayed with 'Buster' to kill the subterranean seedlings and any remaining weeds, to determining whether another flush of emergence was possible.

Emergence was counted again on 15 March 2019. 'Napier' and 'Monti' had the highest second emergence of >1760 seedlings/m^2 (Table 3.12). This is above the recommended minimum 1000 plants/m^2 needed to establish a pure subterranean clover sward (Smetham, 2003). All remaining cultivars, apart from 'Antas', had a seedling population of at least 1440 seedlings/m^2 which would be sufficient for establishment. 'Antas' had a seedling population of 490 plants/m^2, which was less than half the recommended seedling population for a pure sward but more than sufficient if it was in a mixed pasture.

High seedling density is influenced by several factors including: high seed production and burr burial; appropriate levels of hardseed softening based on the environmental conditions; and the ability to avoid 'false strikes' (Nichols *et al.*, 2013a). 'Antas' has a low hardseededness rating of 3, as does 'Narrikup' (Table 2.1). 'Trikkala', 'Denmark' and 'Monti' have a lower hardseededness ratings of 2. The herbicide application may have killed the soft seed of 'Antas' but this seems unlikely to be the only factor as other cultivars that had high seedling populations are also suggested to have a low hardseededness rating. 'Antas' had the lowest burr burial rating of 1, typical of ssp. *brachycalycinum*, which means it has little to no burr burial (Table 2.1). Advantages of burr burial are that the seed is buried in the soil, an ideal environment for germination and establishment, and the seed is less likely to be eaten by grazing animals during summer (Nichols *et al.*, 2013a). All the ssp. *yanninicum* cultivars in this experiment have a burr burial rating of 6 and the ssp. *subterraneum* cultivars range from 5-7. As more 'Antas' seed would be lying on top of the soil it may have germinated with the first rain which would explain the decreased seedling establishment in March. The management of 'Antas' in this experiment was not ideal, with 'Antas' being grazed too hard in early October. The slow regrowth from this grazing may have negatively affected seed production and most of the 'Antas' seed could have germinated in February. However, seed production was not measured so this cannot be known for sure.

3.4.9 Residual control

Imazethapyr had greater control of docks than flumetsulam eight months after application (Table 3.13). This is consistent with Hollaway *et al.* (2006b) who found imazethapyr degraded slower in the soil, with 30% of applied imazethapyr present in the top layer soil after 10 months compared with flumetsulam which was undetectable in some sites. However, poor dock control of flumetsulam may not be just due to soil residue. Flumetsulam supresses docks seedlings rather than killing them (Gawn *et al.*, 2012). This means that dock growth was low during winter and spring but increased later when the effects of the herbicide worn off. Imazethapyr had greater residual control which may improve re-establishment of the pastures the following year, whereas flumetsulam is likely to have no effect.

3.5 Conclusions

Imazethapyr and flumetsulam were equally effective at initially reducing broadleaf weeds with a reduction of ~1000 kg DM/ha for the season. However, imazethapyr had a longer soil residual allowing control of weeds the following March.

'Antas' and 'Napier' were the highest yielding cultivars and the most tolerant to both herbicides. For the remaining cultivars yields were not different. Herbicides had more of an impact on these cultivars at the October harvest, with subterranean clover yields not increasing as they did with 'Antas' and 'Napier' but subterranean clover yields were still improved by both herbicides for the season.

Herbicide treatment had no impact on subterranean clover re-emergence in autumn. 'Antas' had poor seedling re-emergence after a simulated 'false strike', potentially due to poor burr burial. All remaining cultivars had sufficient re-emergent seedling populations for a pure sward.

Based on these results, both herbicides can be recommended for use on emerging subterranean clover. However, imazethapyr has a longer residual and control of broadleaf weeds making it a more suitable choice.

4 WATERLOGGING EXPERIMENTS

4.1 Introduction

The most common subterranean subspecies sown in New Zealand is ssp. *subterraneum*, with ‘Monti’ being the only ssp. *yanninicum* cultivar currently commercially available. Ssp. *subterraneum* is generally more suited to New Zealand hill country conditions as it evolved in a summer dry climate with well drained soils (Katznelson, 1970). However, ssp. *subterraneum* may not be suited to some hill country areas that experience winter waterlogging, such as North Island mudstone soils. Research on naturalized Australian grasslands has shown that ssp. *subterraneum* seed numbers increase as elevation increases and the ssp. *yanninicum* seed numbers increase as elevation decreases and soil gets wetter (Cocks, 1994). This suggests that ssp. *yanninicum* maybe more suited to wet conditions.

The aim of this section is to investigate whether the *yanninicum* subspecies is suitable to be used in winter wet conditions in New Zealand. Therefore, this chapter deals with the Objectives 4-6 of the thesis.

Objective 4: To quantify the yield response of two subterranean clover cultivars, ‘Coolamon’ (ssp. *subterraneum*) and ‘Monti’ (ssp. *yanninicum*) under waterlogging.

Objective 5: To identify any physiological or morphological mechanisms for any differences in response of subterranean clover cultivars to waterlogging.

Objective 6: To quantify plant population and yield response of two ssp. *yanninicum* cultivars and two ssp. *subterraneum* cultivars sown together in a 50:50 subspecies mix under waterlogging.

To do this two experiments were established at Lincoln University to artificially create waterlogged conditions.

4.2 Materials and Methods

4.2.1 Site

A trough experiment was conducted in Iversen 9 at Lincoln University. A second dam experiment was conducted in the headlands of Experiment 1. The headlands were sown on the 24 April 2018 with 10 kg/ha of a ssp. *yanninicum* mix ('Napier' and 'Trikkala') and 10 kg/ha of a ssp. *subterraneum* mix ('Denmark' and 'Narrikup') as described in Section 3.2.5. Climate, soil tests and paddock history were described in Experiment 1 (Section 3.2.2-3.2.4).

4.2.2 Waterlogging treatments

4.2.2.1 Trough experiment

Sixteen plastic troughs (0.33 x 0.45 x 0.20 m) were dug into the ground and refilled with soil. Four of these troughs were bottomless, to create a free draining control treatment. Eight troughs had holes in the bottom to allow some water to drain through. The final four troughs had a sealed bottom to stop water from draining. Subterranean clover seedlings were transplanted from control plots in Experiment 1 into the troughs on the 27 July 2018 (Plate 4.1). These were transplanted into the troughs as intact sections of drill row. Each trough had 0.2 m of drill row (~8-15 plants) of ssp. *yanninicum* ('Monti') and ssp. *subterraneum* ('Coolamon') transplanted. Plots were hand weeded as needed to leave only subterranean clover. Sulphur Super 30 (0,7,0,30) was hand applied to the plots on 28 August 2018 at a rate of 332 kg/ha, or the equivalent of 100 kg S/ha.

Waterlogging treatments began on 31 August 2018. There were four different watering treatments to create a range of soil water levels (Table 4.1). Soil water was measured with a HydroSense II soil moisture sensor (Campbell Scientific, Inc., Utah, USA) to a depth of 10 cm and maintained within the ranges shown in Table 4.1. After 7.5 weeks treatments finished on the 23 October 2018. Holes were made in the bottom of the waterlogging treatments to allow water to drain through before final measurements were taken on 17 December 2018. Thus the treatments were designed to simulate a period of winter waterlogging in late winter/early spring, which would be typical of saturated soils in summer dry regions.

Plate 4.1 Example of trough set up with transplanted subterranean clovers ('Coolamon'-left, 'Monti'-right) for Experiment 2 on 29 August 2018, at Lincoln University, Canterbury.

Table 4.1 Waterlogging treatments, soil water content to 0.1 m and trough type used in Experiment 2 at Iversen 9, Lincoln University.

Treatment	Watering frequency	Soil water (%)	Trough type
Control	-	25-27	Bottomless
Intermediate 1	Once per week	35-37	Holes
Intermediate 2	Three per week	47-49	Holes
Waterlogged	As needed*	50-52	Sealed bottom

* The water level in waterlogging treatments was kept 2-3 cm above the soil level (Plate 4.2).

Plate 4.2 Example of waterlogged treatment used in Experiment 2, left, compared with the control, right, on 5 October 2018 at Iversen 9, Lincoln University, Canterbury, New Zealand.

4.2.2.2 Dam experiment

Two dirt walls were created perpendicular to the I9 paddock fence to create a dam-like structure. The area sloped towards the fence line allowing different degrees of flooding treatments to be achieved (Plate 4.3). The area was flooded for three weeks, from 27 August 2018 until 10 September 2018. Subterranean clover plants were measured on 13 September 2018 from three areas of the dam, with different degrees of flooding, and a rain-fed control as shown in Plate 4.3. Soil water measurements were taken using a HydroSense II soil moisture sensor (Campbell Scientific, Inc., Utah, USA) to a depth of 10 cm from each flooding treatment on 10 September 2018, prior to the dam being drained, and are detailed in Table 4.2.

Table 4.2 Flooding treatments and mean soil water content for the dam in Experiment 2 in Iversen 9, Lincoln University, Canterbury, New Zealand.

Flooding treatment	Soil water (%)
Flooded	51.3±0.13
Intermediate 2	48.2±0.89
Intermediate 1	36.2±0.85
Control	26.5±0.84

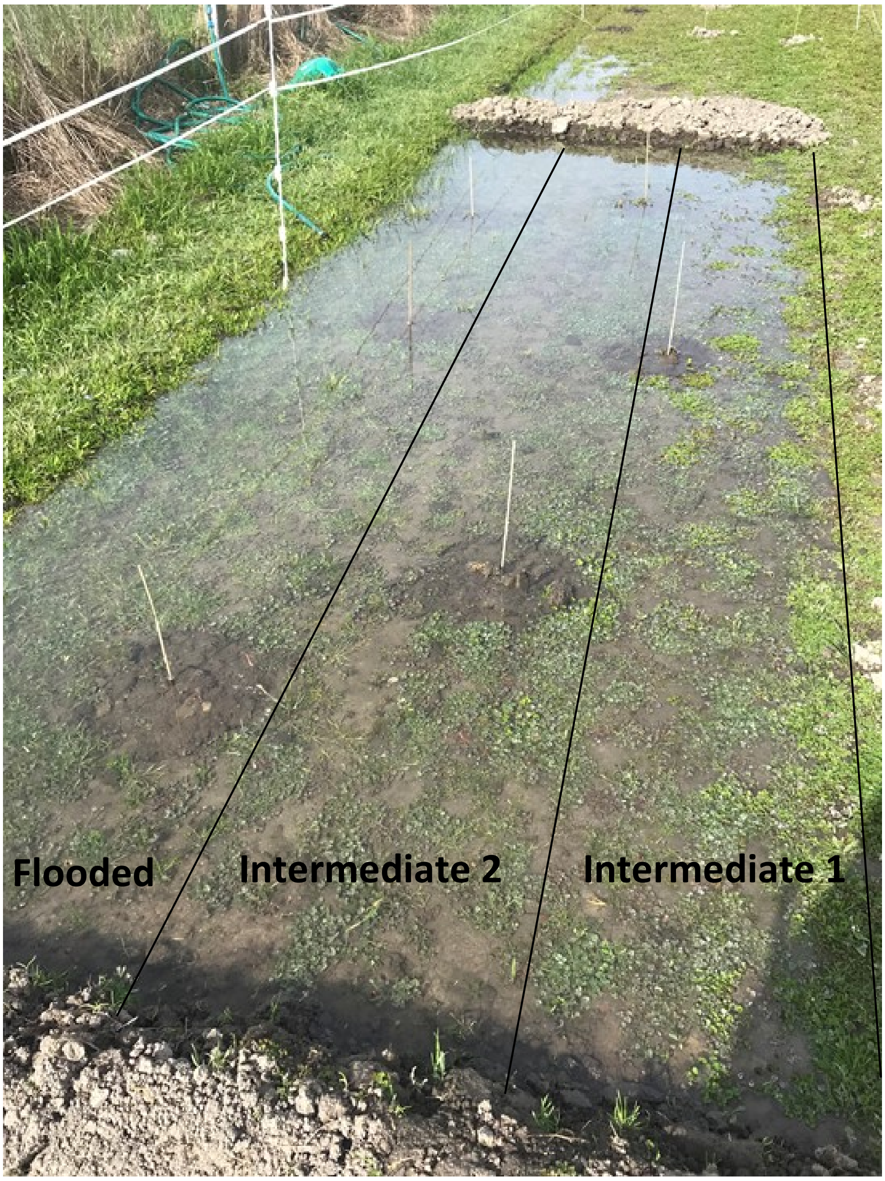

Plate 4.3 Dam created for Experiment 2 with labels showing treatment areas, in Iversen 9, Lincoln University, Canterbury, New Zealand. Note: Control samples were taken from the right of Intermediate 1 and are not pictured.

4.2.3 Trough experiment measurements

4.2.3.1 Dry matter production

Shoots were harvested from just above the soil surface on the 24 October and 17 December 2018. All plants in the 20 cm row were harvested. The whole sample was sorted into subspecies. Samples were dried in an oven at 60°C for 48 hours before weighing. At the end of the experiment (17 December 2018) shoots and roots were harvested and sorted into subspecies. The roots were washed prior to drying. Lateral roots were removed from the tap root for the control and waterlogged treatments and dried and weighed separately. Lateral roots were scored for all treatments on a 0 (no lateral roots) – 5 scale. Nodules were scored 1-3 on colour (1=white, 2=pink) and size (1=small, 3=large) before roots were dried.

4.2.3.2 Relative water content

Relative water content (RWC) was measured on 24 October and 17 December. Two randomly chosen fully unfolded young leaves from each subspecies were harvested per pot and weighed immediately to determine fresh mass (FM). The leaves were then placed in a petri dish and saturated in water overnight at 4^{o}C. Leaves were then patted dry with a paper towel before weighing to determine turgid mass (TM). The samples were then dried at 80^{o}C for 48 hours before being weighed again to determine the dry mass (DM). Relative water content (RWC) was then calculated using Equation 4.1.

Equation 4.1 $$\mathbf{RWC}(\%) = \mathbf{100} \times \frac{(\mathbf{FM-DM})}{(\mathbf{TM\ -DM})}$$

4.2.3.3 Osmotic potential

Leaf tissue was taken at the first harvest, 24 October, to analyse for osmotic potential. A microfuge tube, with a wire sieve placed at the bottom, was filled with randomly selected fully unfolded trifoliate leaves for each cultivar and pot. Samples were then frozen until processing could take place. Prior to centrifuging, open tubes were dipped into liquid nitrogen for 10 seconds to freeze the leaf tissue and then thawed for approximately five minutes. Samples were centrifuged for five minutes to allow extraction of cellular liquid. A sample of 10 µl of extracted liquid was loaded onto a vapour pressure osmometer (Wescor Vapro 5520) to measure total solute concentration. The osmometer was operated in a room at 20^{o}C and was calibrated with standard solution prior to use. A clean test was then performed to check thermocouple contamination levels. Osmotic potential was then calculated with Equation 4.2 where Ψ_s is osmotic potential, RT is the gas constant at 20^{o}C (0.002437 m^3 MPa mol^{-1}) and c_j is the total solute concentration.

Equation 4.2 $$\Psi_{\mathbf{s}} = -\mathbf{RTc_j}$$

4.2.3.4 Photosynthesis

Photosynthesis and stomatal conductance were measured using the LiCor LI-6400XT portable photosynthesis system (LI-COR Biosciences, Inc. Lincoln, Nebraska, USA). The measurements were taken on 23 October 2018 at midday. Air temperature averaged 25°C while the measurements were taking place. Young fully expanded leaves from the upper part of the canopy were chosen for all measurements. The middle leaflet from each leaf from each cultivar was measured per pot. In some of the waterlogged treatments the whole trifoliate leaf was measured due to the small size of these leaves. As the leaves were too small to fill the area of the leaf chamber (6 cm^2), each leaf measured was traced onto paper and cut out with the area of the paper then measured by a leaf area meter. Measurements were later adjusted to account for the leaf area size.

4.2.3.5 Morphological measurements

Visual scores were taken of the percentage of leaf damaged (e.g. chlorosis) and leaf senescence for each treatment. Plants were visually assessed and scored 0 (green)-5 (whole leaf red) for leaf redness.

At the first harvest, petiole length and diameter were measured from two randomly chosen petioles from each cultivar and pot. Petiole diameter was measured with digital callipers.

4.2.4 Dam experiment measurements

Measurements were taken in the dam on 13 September 2018. Three days before measurements were taken watering of the dam stopped to allow time for the soil to drain. Subterranean clover seedlings were harvested at soil height from a 0.1m^2 quadrat, with four samples being taken from different sections of each flooded treatment. Subterranean clover seedlings were separated into sub species. The number of plants per sub species were then counted. Seedlings were then dried in a forced air oven at 60°C for 48 hours before weighing. Shoot dry weight was then divided by plant number to determine average shoot dry weight per plant. Plant diameter was determined by measuring the length of the two longest petioles of the largest plant for each sub species in each sample.

4.2.5 Statistical Analysis

All results were analysed using Genstat 19th edition.

4.2.5.1 Trough experiment

A two way ANOVA with no blocking was used to analyse data from the trough experiment with watering frequency and cultivar as treatments. Means were separated using Fisher's protected LSD with a significance level of α=0.05.

4.2.5.2 Dam experiment

A general ANOVA was used to analyse data from the dam experiment. The treatment structure was set as flooding treatment+subspecies. Blocks were set as flooding treatment*subspecies. Means were separated using Fisher's protected LSD with a significance level of α=0.05.

4.3 Results

4.3.1 Trough experiment

4.3.1.1 Dry matter production

At the first harvest, shoot DW was affected (P=0.003) by the cultivar*water interaction (Figure 4.1). 'Coolamon' watered 1x a week had the highest shoot DW of 1330 g DM/m^2 out of all of the 'Coolamon' treatments. 'Coolamon' watered 3x a week had a reduced shoot DW of 710 g DM/m^2 compared with the control. Waterlogged 'Coolamon' had the lowest shoot DW of 230 g DM/m^2 or 83% of its highest yielding treatment.

In contrast, the highest shoot DW for 'Monti' was achieved when watered 3x a week, averaging 1430 g DM/m^2, although this was not different to 'Monti' watered 1x a week. The 'Monti' control and waterlogged 'Monti' did not differ from each other and had shoot DWs of 835 g DM/m^2 and 1080 g DM/m^2. Waterlogged 'Monti' was reduced by 46% compared with the 3x a week watering treatment.

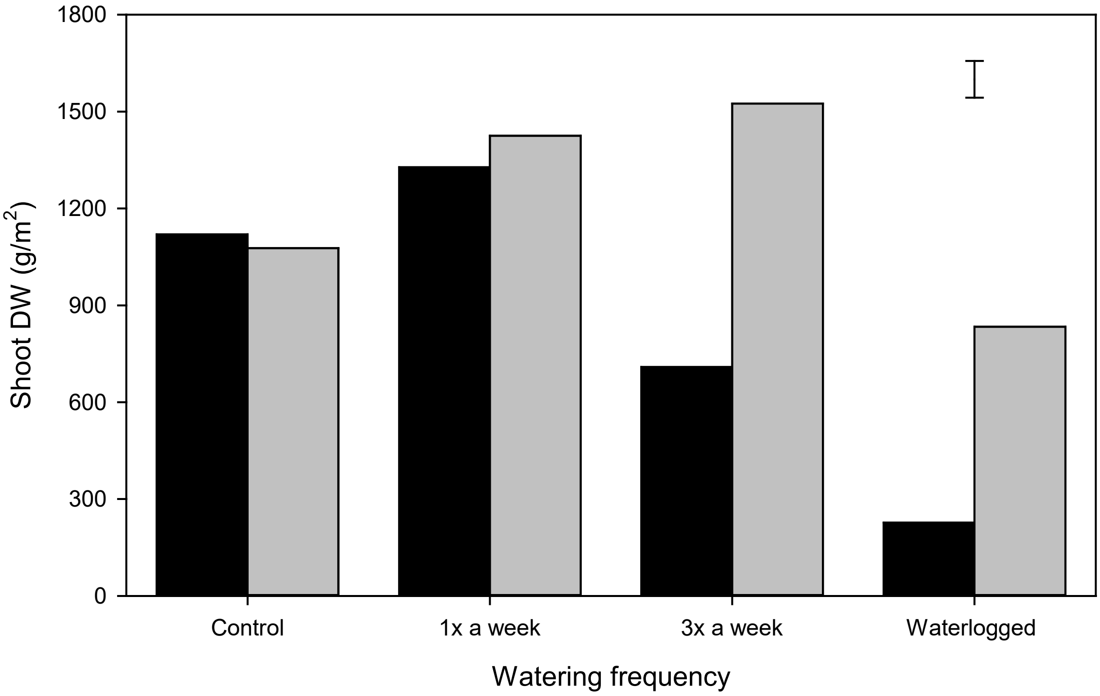

Figure 4.1 Mean shoot dry weight (DW) of two subterranean clover cultivars, 'Coolamon' (■) and 'Monti' (■), on 24 October 2018 across four watering treatments at Iversen 9, Lincoln University, Canterbury, New Zealand. Error bar is the SEM for watering frequency*cultivar interaction.

There was a cultivar effect ($P<0.001$) at the second harvest (Figure 4.2) two months after treatments were finished. 'Monti' had a higher average shoot DW across all four watering treatments of 302 g/m^2 compared with 149 g/m^2 for 'Coolamon'.

Watering frequency also had an effect ($P<0.001$) on shoot DW. The shoot DW of the waterlogged treatments was reduced by ~62% of the other three treatments to 94 g/m^2, showing an ongoing loss in yield from the earlier waterlogging.

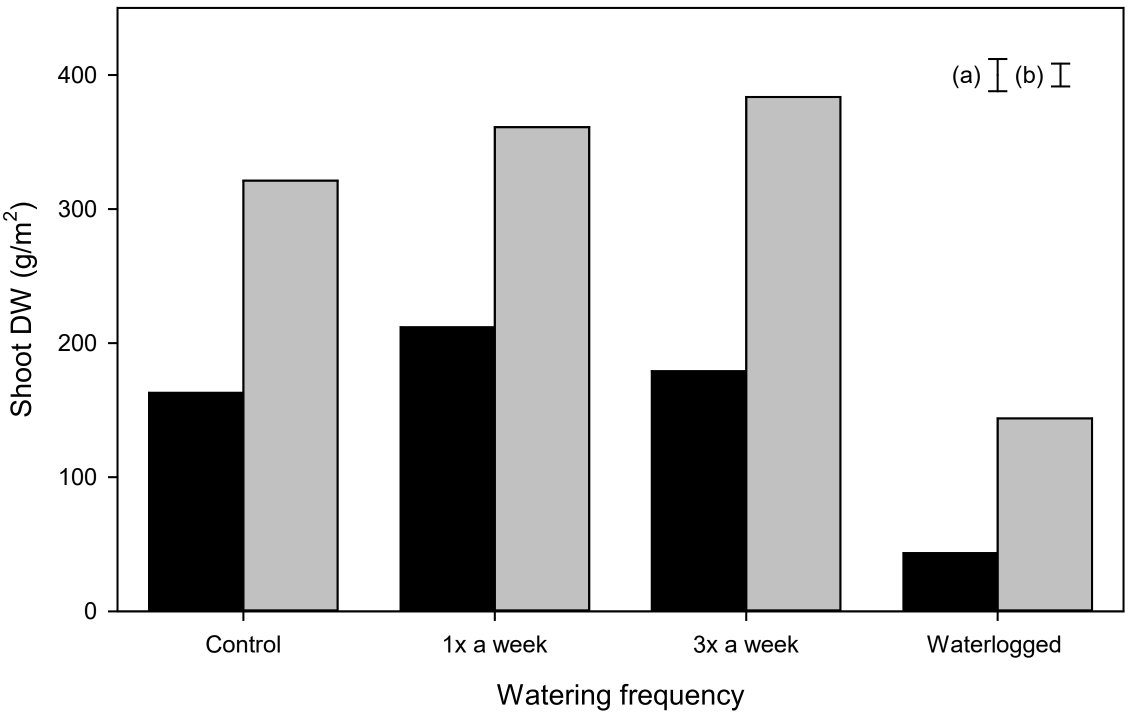

Figure 4.2 Mean shoot dry weight (DW) of two subterranean clover cultivars, 'Coolamon' (■) and 'Monti' (■), on 17 December 2018 after four watering treatments at Iversen 9, Lincoln University, Canterbury, New Zealand. Error bars are the SEM for (a) the main effect of watering frequency; (b) the main effect of cultivar.

4.3.1.2 Root dry weights

There was a cultivar*water level interaction (P=0.007) for root DW (Figure 4.3). Waterlogged 'Monti' had the highest root DW of all treatments, apart from the 'Monti' control, of 47 g/m^2. There was no difference between the 'Coolamon' treatments and 'Monti' watered 1x and 3x a week with root DW ranging between 19-29 g/m^2.

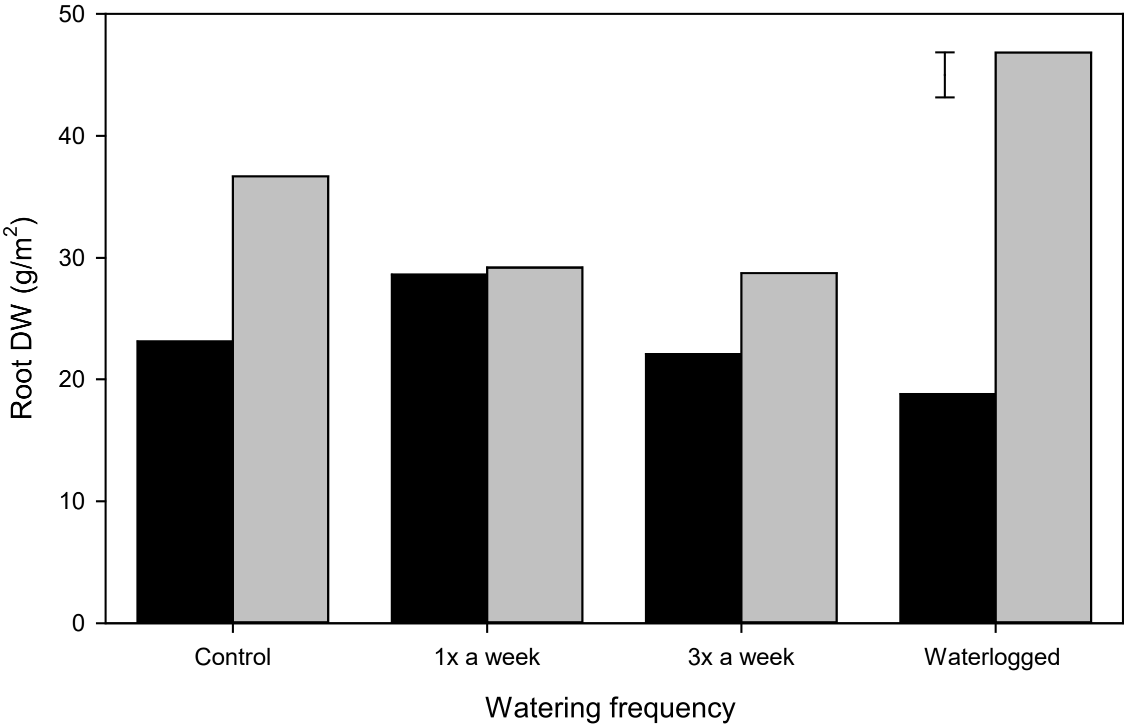

Figure 4.3 Mean root dry weights (DW) of two subterranean clover cultivars, 'Coolamon' (■) and 'Monti' (■), on 17 December 2018 after four watering treatments at Iversen 9, Lincoln University, Canterbury, New Zealand. Error bar is the SEM for watering frequency*cultivar interaction.

The results of the taproot and lateral root components for the control and waterlogged treatments are shown in Figure 4.4. 'Monti' had a larger ($P<0.001$) tap root than 'Coolamon' of 20 g/m^2 compared with 10 g/m^2. Watering frequency also had an effect ($P<0.001$) on taproot DW. Waterlogging reduced the taproot DW by 33% compared with the control to 12 g/m^2.

Lateral root DW was affected ($P=0.009$) by the cultivar*water level interaction (Figure 4.4). Waterlogged 'Monti' had the highest lateral root DW of 31 g/m^2, an increase of 60% compared with its control. There was no difference between either the control or the waterlogged 'Coolamon', averaging 11.5 g/m^2.

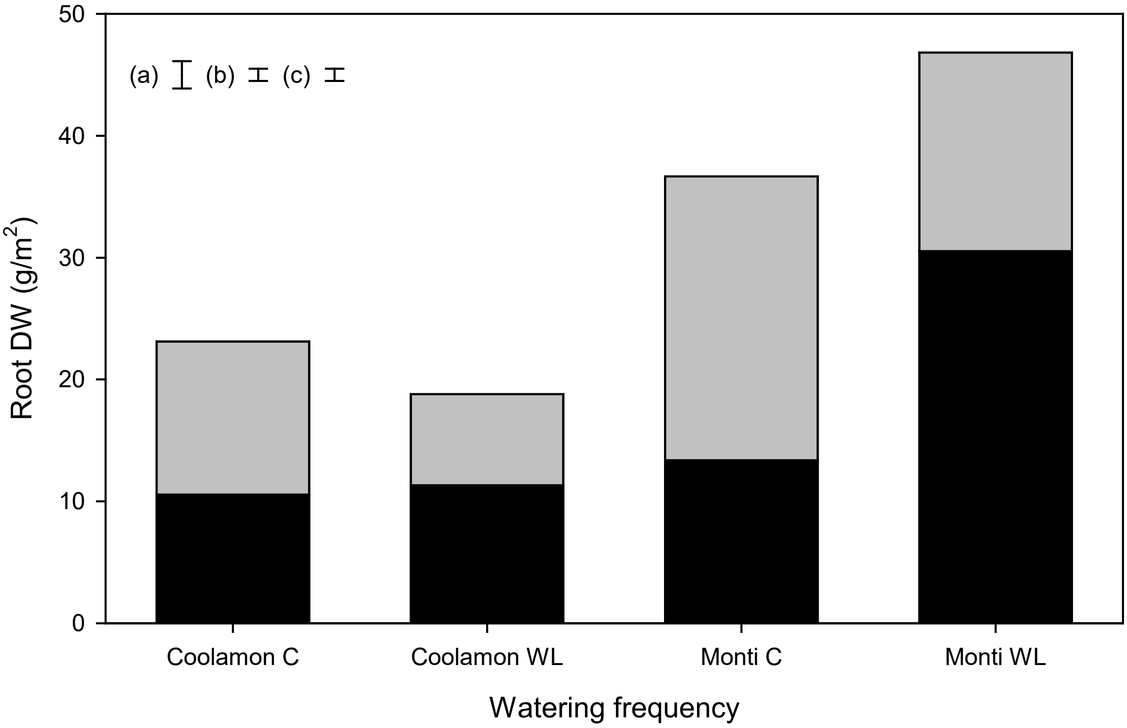

Figure 4.4 Mean taproot (■) and lateral root (■) dry weights (DW) of two subterranean clover cultivars, 'Coolamon' and 'Monti', on 17 December 2018 after two watering treatments at Iversen 9, Lincoln University, Canterbury, New Zealand. C – control, WL – waterlogging. Error bars are the SEM for (a) watering frequency*cultivar interaction for lateral root DW; (b) the main effect of watering frequency on taproot DW; (c) the main effect of cultivar on taproot DW.

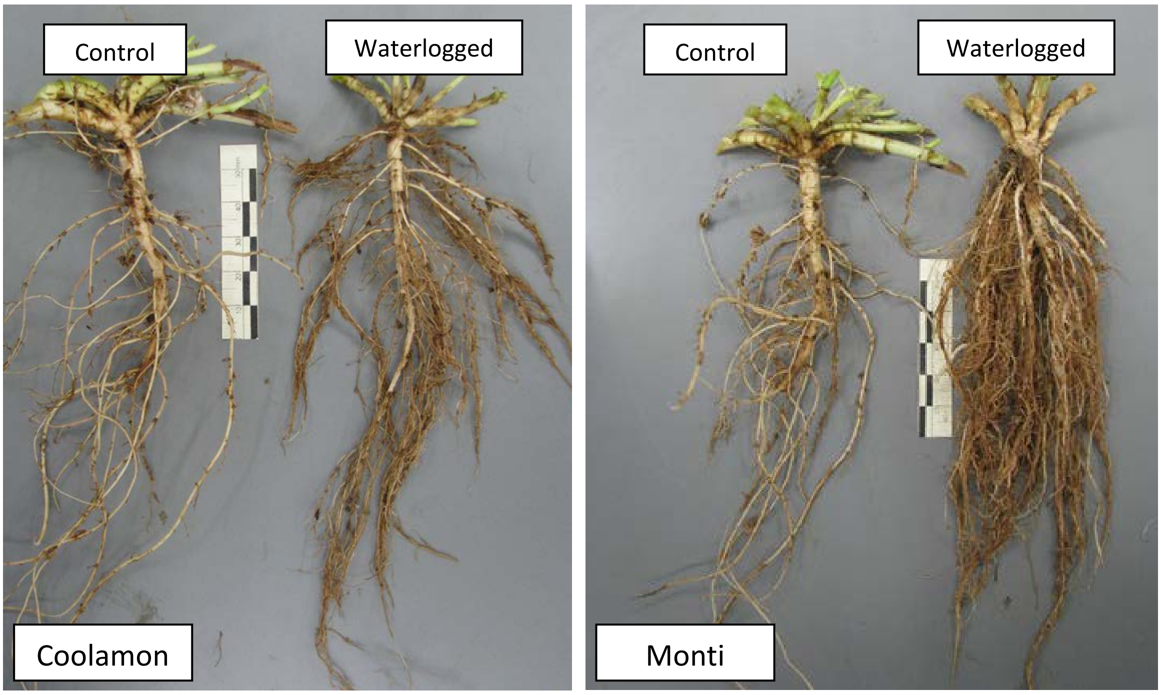

Plate 4.4 'Coolamon' and 'Monti' subterranean clover roots after waterlogging at Iversen 9, Lincoln University, Canterbury, New Zealand. Left= 'Coolamon', right= 'Monti'.

A visual score of all four watering treatments showed only watering frequency had an effect (P=0.003) on the lateral root score (Table 4.3). The waterlogged treatment had the highest lateral root score of 4.00. The remaining watering treatments ranged between 1.88-2.88.

Table 4.3 Mean lateral root score of two subterranean clover cultivars across four watering treatments on 18 October 2018 at Iversen 9, Lincoln University, Canterbury, New Zealand.

Watering frequency	'Coolamon'	'Monti'	Mean
Control	2.50	3.25	2.88_b
1x a week	1.75	2.00	1.88_b
3x a week	2.00	2.25	2.13_b
Waterlogged	3.75	4.25	4.00_a
P value – WF		0.003	
P value – CV		0.195	
P value – WF*CV		0.973	
SEM – WF		0.375	

WF – watering frequency, CV – cultivar. Lateral roots scored 0 (no lateral roots) – 5.

4.3.1.3 Taproot length and diameter

The results of the taproot diameter are show in Figure 4.5. 'Monti' had a larger ($P<0.001$) taproot diameter, averaging 4.76 mm across all four watering treatments, than 'Coolamon' which averaged 3.35 mm. Watering frequency had no effect ($P=0.462$) on taproot diameter.

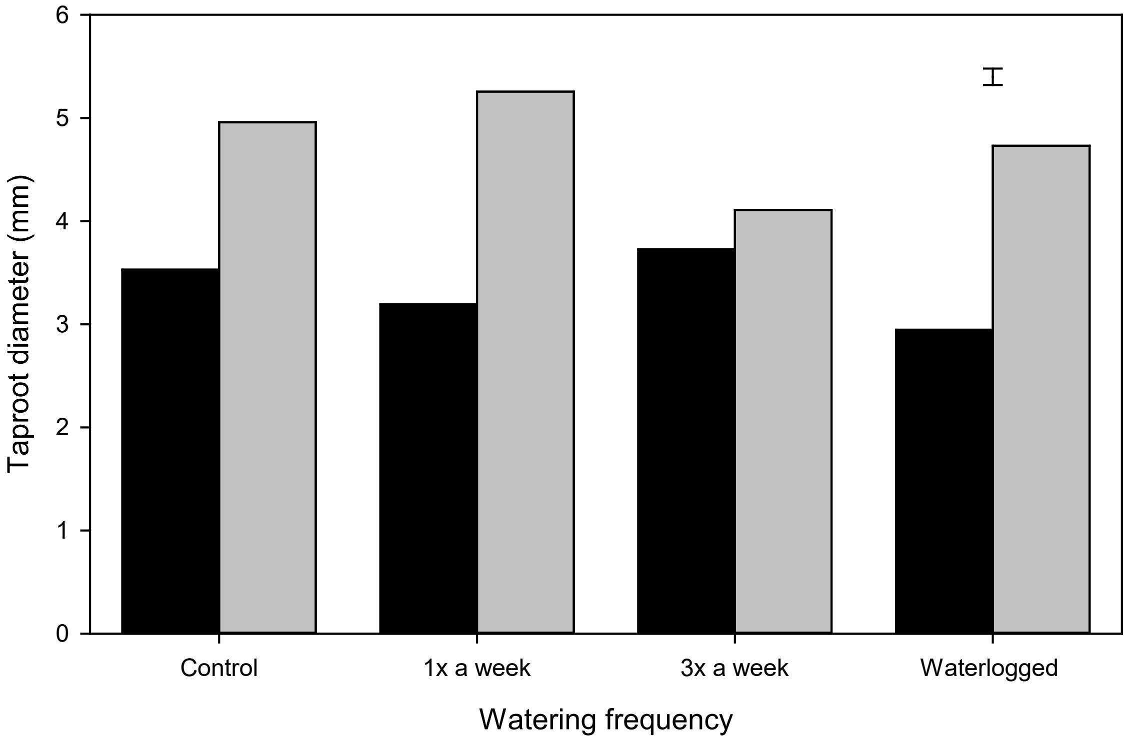

Figure 4.5 Mean taproot diameter of two subterranean clover cultivars, 'Coolamon' (■) and 'Monti' (■), on 17 December 2018 after four watering treatments at Iversen 9, Lincoln University, Canterbury, New Zealand. Error bar is the SEM for the main effect of cultivar.

There was no effect ($P=0.335$) of cultivar on taproot length (Figure 4.6). However, watering frequency had an effect ($P<0.001$) on the taproot length of the subterranean clovers. Subterranean clover watered 1x a week had the longest taproot, averaging 21.4 cm. Subterranean clover watered 3x a week had a longer taproot (16.7 cm) than clover that was waterlogged (10.6 cm).

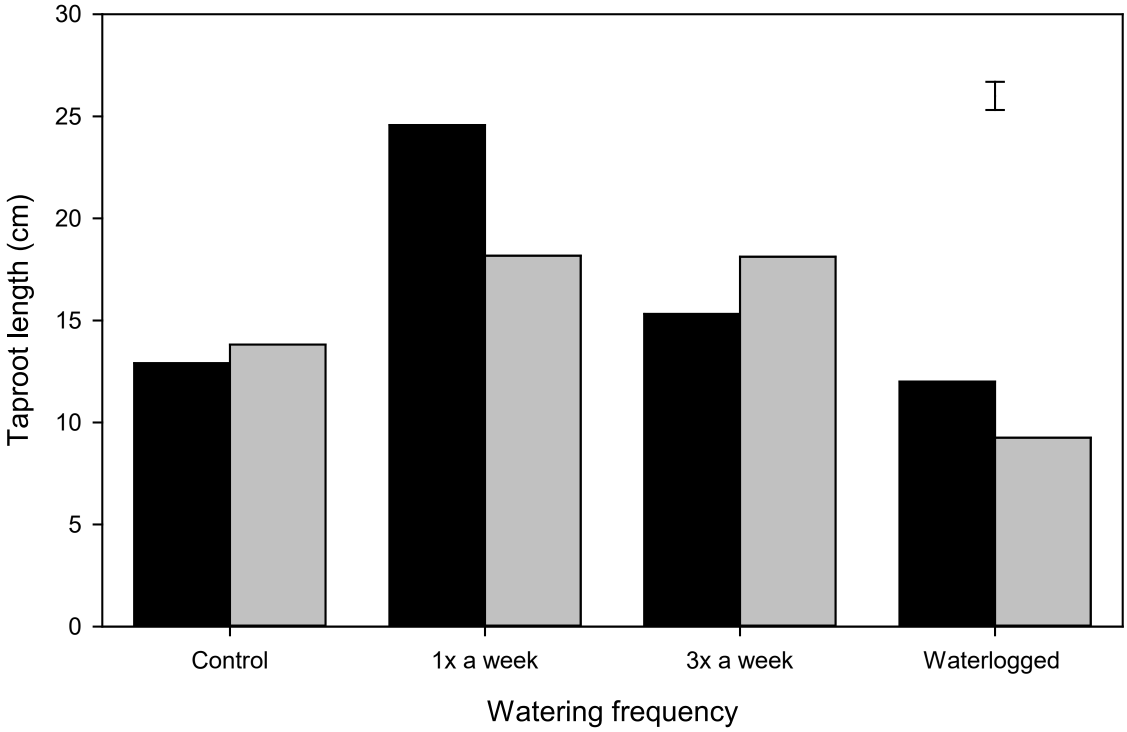

Figure 4.6 Mean taproot length of two subterranean clover cultivars, 'Coolamon' (■) and 'Monti' (■), on 17 December 2018 after four watering treatments at Iversen 9, Lincoln University, Canterbury, New Zealand. Error bar is the SEM for main effect of watering frequency.

4.3.1.4 Nodule colour and size

Nodule colour score was darkest (P=0.008) in the waterlogged (1.50) and 3x a week (1.75) treatments (Table 4.4). The 1x a week watering treatment had the lowest nodule colour score of 1.00.

There was a watering frequency*cultivar interaction (P=0.005) for nodule size (Table 4.4). All four of the 'Monti' watering treatments and waterlogged 'Coolamon' had the highest nodule size score ranging between 2.00-2.25. 'Coolamon' watered 1x a week had the lowest nodule size score of 1.00.

Table 4.4 Nodule colour and size scores of two subterranean clover cultivars, 'Coolamon' and 'Monti', 17 December 2018 after four watering treatments at Iversen 9, Lincoln University, Canterbury, New Zealand.

Watering frequency	Nodule colour			Nodule size	
	'Coolamon'	'Monti'	Average	'Coolamon'	'Monti'
Control	2.50	2.00	2.25_{bc}	1.50_{c}	2.00_{ab}
1x a week	2.00	2.00	2.00_{c}	1.00_{d}	2.25_{a}
3x a week	2.75	2.75	2.75_{a}	1.75_{bc}	2.00_{ab}
Waterlogged	2.75	2.25	2.50_{ab}	2.00_{ab}	2.00_{ab}
P value – WF	0.008			0.141	
P value – CV	0.096			<0.001	
P value – WF*CV	0.410			0.005	

WF- watering frequency, CV – cultivar. Nodule size scored 1 (small) – 3 (large). Nodule colour scored 1 (white) – 3 (pink/red). SEM of main effect of WF for nodule colour = 0.1443. SEM of WF*CV interaction for nodule size = 0.2282.

Plate 4.5 Nodules on 'Coolamon' control roots from Experiment 2 at Iversen 9, Lincoln University, Canterbury, New Zealand. Scored 2 for nodule size and 2 for nodule colour.

4.3.1.5 Petiole length and diameter

The results for petiole length are shown in Figure 4.7. Waterlogged plants had the shortest ($P<0.001$) petiole lengths averaging 8.1 cm or nearly 50% shorter than the control. The remaining three watering treatments ranged between 15.2-15.9 cm. Cultivar also had an effect ($P<0.001$) on petiole length. 'Monti' had longer petioles (16.0 cm) compared with 'Coolamon' (11.4 cm).

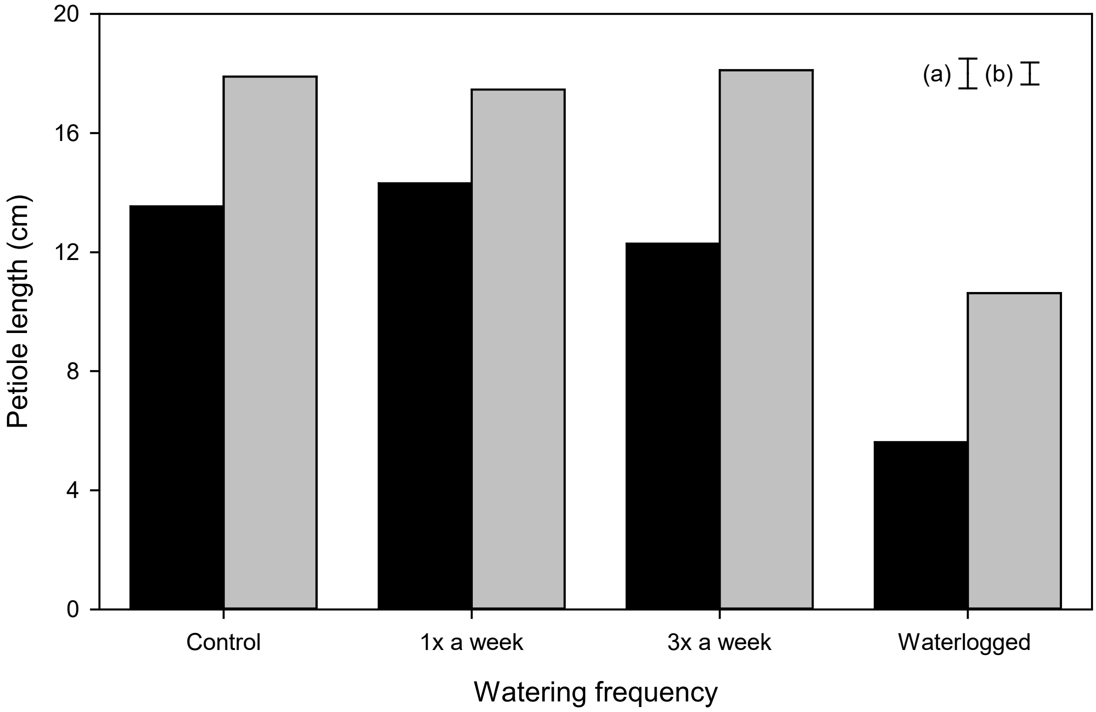

Figure 4.7 Mean petiole length of two subterranean clover cultivars, 'Coolamon' (■) and 'Monti' (■), on 24 October 2018 across four watering treatments at Iversen 9, Lincoln University, Canterbury, New Zealand. Error bars are the SEM for (a) the main effect of watering frequency; (b) the main effect of cultivar.

Watering frequency had no effect ($P=0.414$) on petiole diameter (Figure 4.8). 'Monti' had a larger ($P=0.026$) petiole diameter, which averaged 1.91 mm across the four watering treatments, compared with 1.87 mm for 'Coolamon'.

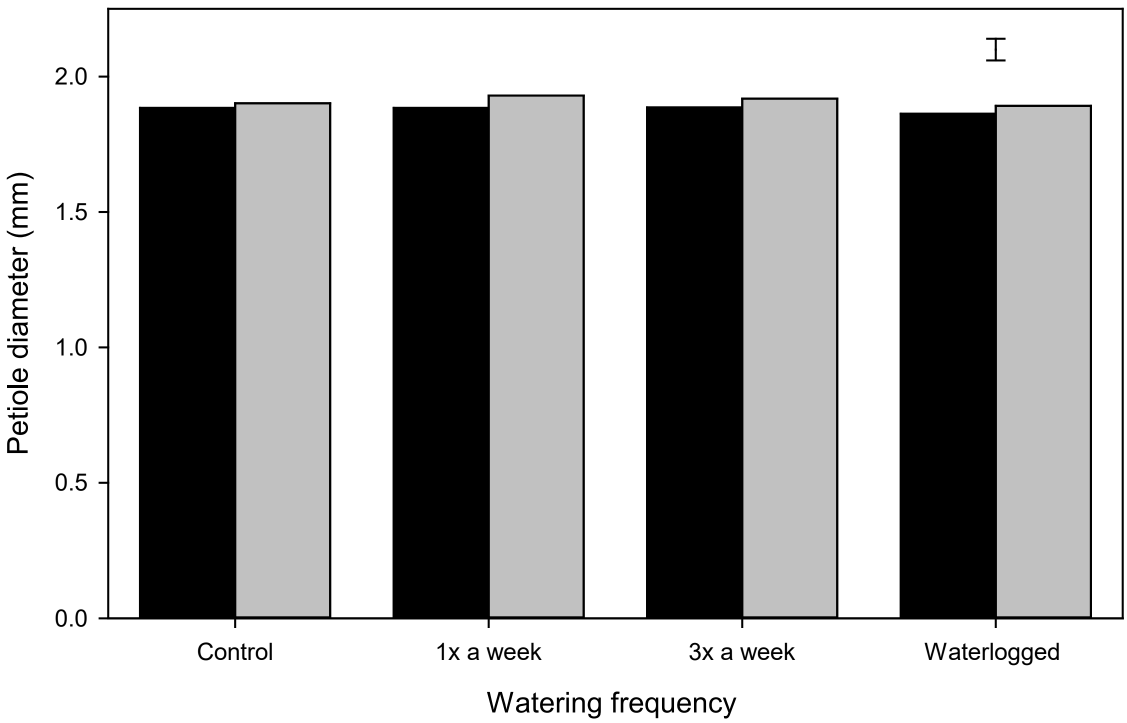

Figure 4.8 Mean petiole diameter of two subterranean clover cultivars, 'Coolamon' (■) and 'Monti' (■), on 24 October 2018 across four watering treatments at Iversen 9, Lincoln University, Canterbury, New Zealand. Error bar is the SEM for the main effect of cultivar.

4.3.1.6 Relative water content

The results of RWC for the first harvest are shown in Figure 4.9. Cultivar (P=0.221) and watering frequency (P=0.546) had no effect on RWC, which ranged from 84.2-90.1%.

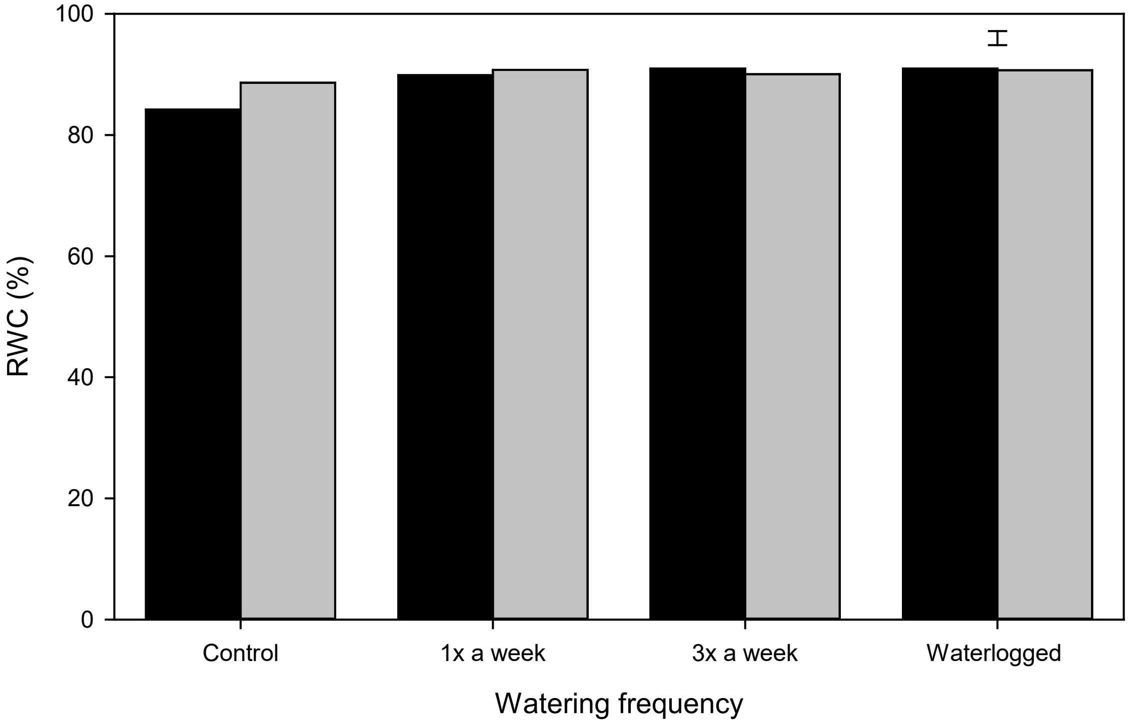

Figure 4.9 Mean relative water content (RWC) of two subterranean clover cultivars, 'Coolamon' (■) and 'Monti' (■), on 24 October 2018 across four watering treatments at Iversen 9, Lincoln University, Canterbury, New Zealand. Error bar is the SEM for watering frequency*cultivar interaction.

As with the first harvest, cultivar (P=0.354) and watering frequency (P=0.860) had no effect on RWC on 17 December 2018 (Figure 4.10). RWC ranged from 73.7-94.6% across the treatments.

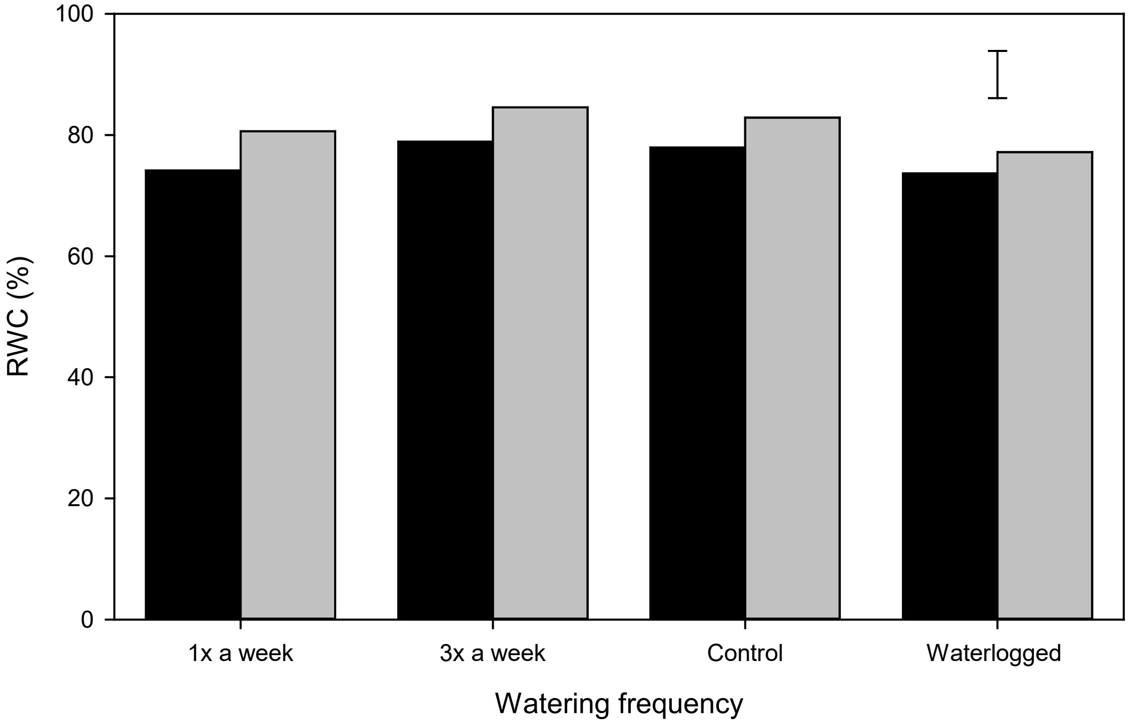

Figure 4.10 Mean relative water content (RWC) of two subterranean clover cultivars, 'Coolamon' (■) and 'Monti' (■), on 17 December 2018 after four watering treatments at Iversen 9, Lincoln University, Canterbury, New Zealand. Error bar is the SEM for watering frequency*cultivar interaction.

4.3.1.7 Osmotic potential

Waterlogged subterranean clover had the highest (P=0.027) osmotic potential of -0.94 MPa (Figure 4.11). The remaining three treatments averaged -1.07 MPa. There was no difference (P=0.928) in osmotic potential between the cultivars.

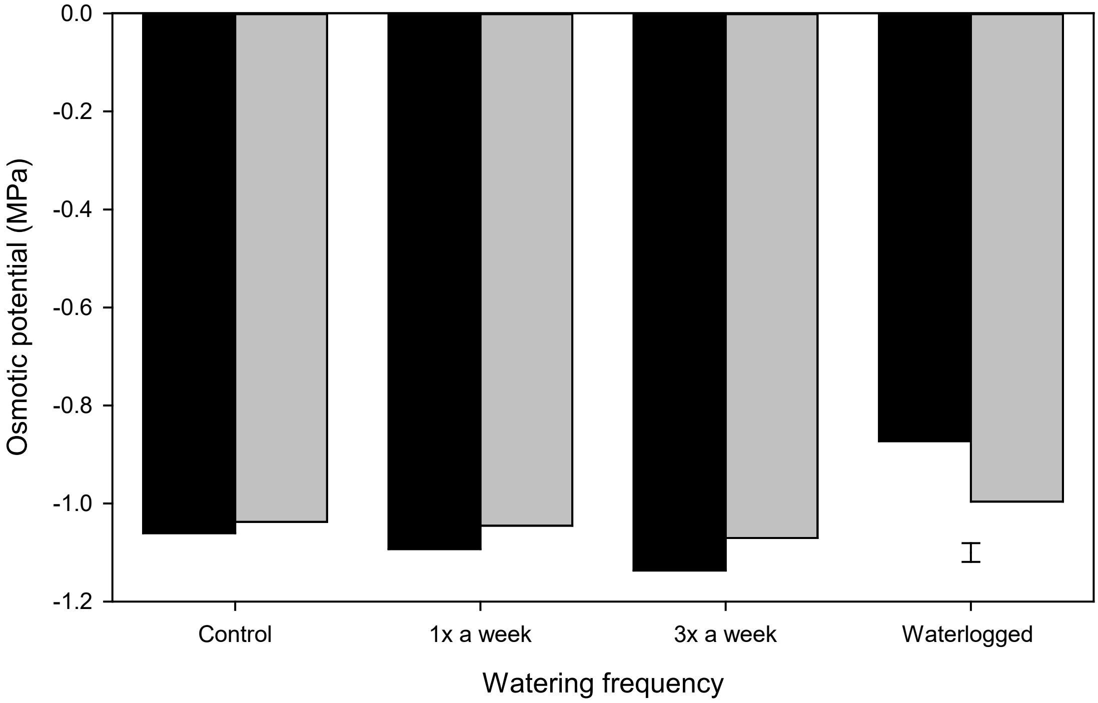

Figure 4.11 Mean osmotic potential of two subterranean clover cultivars, 'Coolamon' (■) and 'Monti' (■), on 24 October 2018 across four watering treatments at Iversen 9, Lincoln University, Canterbury, New Zealand. Error bar represents SEM for main effect of watering frequency.

4.3.1.8 Photosynthesis and stomatal conductance

Photosynthetic rate was affected ($P<0.001$) by watering frequency, with the waterlogged treatments having the lowest photosynthetic rate of 15.0 µmol CO_2 m^{-2} s^{-1} or 42% of the control (Table 4.5). There was no difference between the remaining treatments. 'Monti' had a higher ($P=0.018$) photosynthetic rate of 24.5 µmol CO_2 m^{-2} s^{-1} compared with 21.0 µmol CO_2 m^{-2} s^{-1} for 'Coolamon'. There was an indication ($P=0.057$) of a cultivar*watering frequency interaction. The LSD (5.643) suggests the photosynthetic rate of 10.8 µmol CO_2 m^{-2} s^{-1} for waterlogged 'Coolamon' was lower than the photosynthetic rate of 19.1 µmol CO_2 m^{-2} s^{-1} for waterlogged 'Monti'.

There was no effect of cultivar ($P=0.127$) or watering frequency ($P=0.880$) on stomatal conductance which averaged 0.821 mol H_2O m^{-2} s^{-1} among treatments. However, the LSD for cultivar*watering frequency (0.3768) suggests that waterlogged 'Coolamon' had a lower stomatal conductance of 0.580 mol H_2O m^{-2} s^{-1} than waterlogged 'Monti' (0.971 mol H_2O m^{-2} s^{-1}).

Table 4.5 Photosynthesis and stomatal conductance of two subterranean clover cultivars, 'Coolamon' and 'Monti', on 23 October 2018 across four watering treatments at Iversen 9, Lincoln University, Canterbury, New Zealand.

Watering frequency	Photosynthesis (μmol CO_2 m^{-2} s^{-1})			Conductance (mol H_2O m^{-2} s^{-1})	
	'Coolamon'	'Monti'	Average	'Coolamon'	'Monti'
Control	26.6	24.5	25.5_a	0.883	0.725
1x a week	24.1	25.8	24.9_a	0.733	0.920
3x a week	22.5	28.5	25.5_a	0.799	0.956
Waterlogged	10.8	19.1	15.0_b	0.580	0.971
Average	21.0_b	24.5_a			
P value –WF		<0.001		0.880	
P value – CV		0.018		0.127	
P value – WF*CV		0.057		0.229	

WF –watering frequency, CV – cultivar. SEM for the main effect of WL on photosynthesis = 1.367. SEM for the main effect of CV on photosynthesis = 0.967. SEM for the WL*CV interaction on photosynthesis = 1.933

4.3.1.9 Leaf redness

For the first harvest, there was a watering frequency*cultivar interaction (P<0.001) for leaf redness (Table 4.6). Waterlogged 'Monti' had the highest leaf redness score of 4.00. The other remaining treatments showed no redness at 1.00-1.13.

The results for the second harvest followed a similar pattern (Table 4.6). Waterlogged 'Monti' had the highest (P<0.001) leaf redness score of 2.75 compared with 1.00 for the other treatments.

Table 4.6 Leaf redness score of two subterranean clover cultivars, 'Coolamon' and 'Monti', on 24 October 2018 and 17 December 2018 after four watering treatments at Iversen 9, Lincoln University, Canterbury, New Zealand.

Watering frequency	24 October 2018		17 December 2018	
	'Coolamon'	'Monti'	'Coolamon'	'Monti'
Control	1.00_b	1.00_b	1.00_b	1.00_b
1x a week	1.00_b	1.00_b	1.00_b	1.00_b
3x a week	1.00_b	1.00_b	1.00_b	1.00_b
Waterlogged	1.13_b	4.00_a	1.00_b	2.75_a
P value – WF	<0.001		<0.001	
P value – CV	<0.001		<0.001	
P value – WF*CV	<0.001		<0.001	
SEM – WF*CV	0.2500		0.0884	

Leaf redness scored 1 (no colour change) – 5 (whole leaf red).

Plate 4.6 Leaf redness of 'Monti' subterranean clover (left –waterlogged with leaf redness score of 4, right – control with a leaf redness score of 1) on 24 October 2018 at I9, Lincoln University, Canterbury, New Zealand.

4.3.1.10 Leaf damage and scenscence

Waterlogged 'Monti' had less (P=0.033) leaf damage at 15±1.67% compared with 24±1.67% for waterlogged 'Coolamon'. Waterlogged 'Monti' also had less (P=0.011) leaf senescence which was 2.5±2.27% compared with 16±2.27% for waterlogged 'Coolamon'. All other treatments had no senescence or leaf damage at the end of the watering period.

4.3.2 Dam experiment

4.3.2.1 Subterranean clover population

The waterlogged section of the dam had the lowest (P=0.004) subterranean clover population of 131 plants/m^2 (Figure 4.12). Population increased as flooding decreased, with the Intermediate 2 treatment having a population of 200 plants/m^2. There was no difference between the Intermediate 1 and control treatment which averaged 167 plants/m^2.

Statistical limitations in replications prevented the analysis of flooding treatment*subspecies but it appears the population of ssp. *subterraneum* decreased more rapidly than ssp. *yanninicum* in response to flooding. The ssp. *subterraneum*:ssp. *yanninicum* ratio was equal for the control and Intermediate 1 treatment. However, there were more ssp. *yanninicum* in the Intermediate 2 and flooded treatments, a difference of 35 and 28 plants/m^2, respectively.

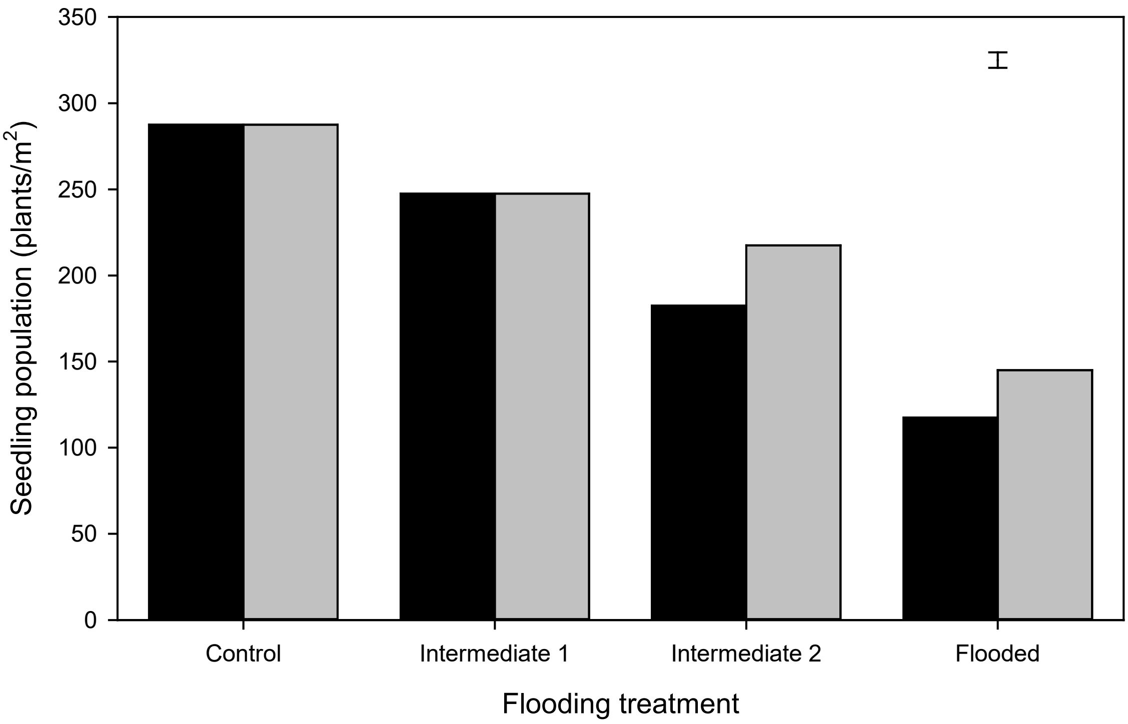

Figure 4.12 Seedling population (plants/m^2) of two subterranean clover subspecies, ssp. *subterraneum* (■) and ssp. *yanninicum* (■), across four flooding treatments on 13 September 2018 at Iversen 9, Lincoln University, Canterbury, New Zealand. Error bar is the SEM for the main effect of flooding treatment.

4.3.2.2 Dry weight

The shoot DW of ssp. *yanninicum* averaged 221 mg/plant across all four treatments (Figure 4.13). This was higher (P=0.037) than the shoot DW of ssp. *subterraneum* (136 mg/plant). Flooding treatment had no effect (P=0.091) on shoot DW.

The flooding appeared to have reduced the shoot DW of ssp. *subterraneum* by 102 mg/plant compared with the control. The shoot DW of flooded ssp. *yanninicum* was not reduced compared with the control.

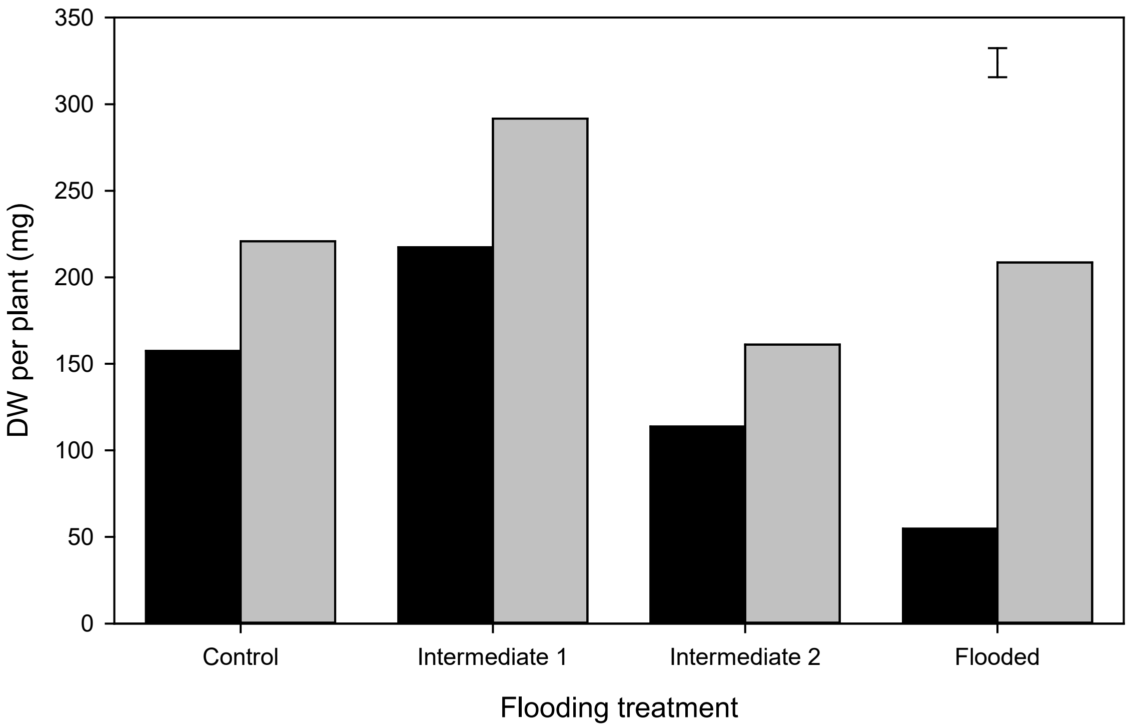

Figure 4.13 Dry weight per plant (mg) of two subterranean clover subspecies, ssp. *subterraneum* (■) and ssp. *yanninicum* (■), across four flooding treatments on 13 September 2018 at Iversen 9, Lincoln University, Canterbury, New Zealand. Error bar is the SEM for the main effect of subspecies.

4.3.2.3 Plant diameter

Plant diameter was highest (P=0.019) for ssp. *yanninicum,* averaging 18.0 cm across the four treatments (Table 4.7). Plant diameter averaged 15.2 cm for ssp. *subterraneum*. There was an indication (P=0.078) that flooding treatment had an effect on plant diameter. The

Intermediate 2 had the longest plant diameter, averaging 18.8 cm, compared with all treatments but the flooded treatment.

Table 4.7 Plant diameter (cm) of two subterranean clover subspecies, ssp. *subterraneum* and ssp. *yanninicum*, across four flooding treatments on 13 September 2018 at Iversen 9, Lincoln University, Canterbury, New Zealand.

Treatment	Ssp. *subterraneum*	Ssp. *yanninicum*
Control	14.3	16.7
Intermediate 1	14.8	17.1
Intermediate 2	18.0	19.8
Flooded	13.9	18.4
Average	15.2	18.0
P value – Ssp.	0.019	
P value – FT	0.078	

FT = flooding treatment. SEM for ssp. = 0.423, SEM for FT = 0.598

4.4 Discussion

4.4.1 Dry matter production

'Monti' was more tolerant to waterlogging than 'Coolamon'. 'Monti' had a higher shoot dry weight than 'Coolamon' at the end of the waterlogging period for the waterlogged and 3x a week treatments (Figure 4.1). This is consistent with findings from Francis and Devitt (1969) who found the ssp. *yanninicum* had a higher yield under waterlogging for 21 days compared with ssp. *subterraneum*. There was no difference between the yield of the two cultivars for the control and 1x a week treatment. 'Monti' had the highest yield of ~1400±162 g DM/m^2 when watered 1x or 3x a week. This is in comparison with 'Coolamon' which had the highest yield of 1330±162 g DM/m^2 when watered 1x a week but this reduced by 610±162 g DM/m^2 when watered 3x a week. These results were supported by the dam experiment which showed that ssp. *subterraneum* had a greater reduction in shoot DW per plant after three weeks of waterlogging compared with ssp. *yanninicum* (Figure 4.13). 'Monti' that had been waterlogged was visually healthier than 'Coolamon' at the end of the waterlogging period (Section 4.3.1.10).

A contributing factor to the waterlogging tolerance of 'Monti' was its ability to increase root production when waterlogged. 'Monti' that was waterlogged had an increase in root dry matter compared with the 1x a week and 3x a week treatments but this was not different to the control (Figure 4.3). ‘Coolamon’ had no difference in root dry matter among the four treatments. Waterlogged ‘Monti’ had an increase in lateral root DW of 60% compared with the control (Figure 4.4). Lateral root and taproot DW were only measured for the control and waterlogged treatment but visual scores of lateral roots showed no difference between the control and the other two treatments (Table 4.3). This is in contrast to Francis and Devitt (1969) who found ssp. *yanninicum* root growth to decrease by 26% under waterlogging conditions. The increase in lateral root growth in this experiment may be due to the long period of waterlogging (7.5 weeks) allowing the adaption of increased lateral root production. The waterlogging in the Francis and Devitt (1969) experiment was for 21 days which may not have given the ssp. *yanninicum* enough time to increase lateral root production. Increased lateral root production is one mechanism by which plants adapt to waterlogged conditions (Armstrong *et al.*, 1991). Lateral roots at or near the soil surface increase the amount of oxygen absorbed by the roots. Waterlogged ‘Monti’ was observed to have produced lateral roots above the soil surface (Plate 4.7) which has previously been seen in ssp. *yanninicum* cultivars (Francis and Devitt, 1969). Other waterlogging tolerant *Trifolium* species, such as Persian clover, also respond to waterlogging by lateral root formation (Gibberd and Cocks, 1997). The taproot DW of waterlogged ‘Monti’ decreased compared with the control. This may be due to ‘Monti’ putting increased resources in the production of lateral roots at and near the surface of the soil rather than increasing tap root length and size.

‘Coolamon’ that was waterlogged had no increase in lateral roots compared with its control but its taproot DW decreased by 33% (Figure 4.4). This is consistent with Francis and Devitt (1969) who found that ssp. *subterraneum* root growth to decrease by 46% under waterlogging conditions for 21 days. ‘Coolamon’ did not increase lateral root production therefore had a low shoot DW when waterlogged. This reduction in taproot DW was probably due to the inability of the roots to absorb adequate oxygen as hypoxic conditions can kill roots (Armstrong *et al.*, 1991).

Plate 4.7 Surface roots produced by 'Monti' subterranean clover after eight weeks of waterlogging on 24 October 2018 at Iversen 9, Lincoln University, Canterbury, New Zealand.

Neither subterranean clover subspecies had fully recovered from waterlogging eight weeks after treatments had finished. The shoot DW of the waterlogged treatments was reduced by 62% compared with the control (Figure 4.3). It seems likely that the damage done to the 'Coolamon' roots was too great to recover from within eight weeks. However, 'Coolamon' watered 3x a week, which had a low yield in the first harvest, did recover and had the same yield as its control and 1x a week treatments. This shows that recovery was possible if the waterlogging was as extreme as imposed by the 3x a week treatment. Despite the waterlogging 'Monti' had a high root DM and most of this DM came from lateral roots near the soil surface with a reduced taproot length. This would limit the ability of 'Monti' to uptake water from the lower soil levels as the soil dried out towards summer unless it grew a deeper root once the waterlogging was removed.

For both cultivars the subterranean clover watered 1x a week had the longest taproots (21±1.38 cm) probably due to the increase in water allowing more growth (Figure 4.6).

However, as watering increased the taproot length decreased to 17±1.38 cm for the 3x a week watering treatment and 11±1.38 cm for the waterlogged treatment. This may be due to the plant adjusting to keep its taproot out of the lower anaerobic layers of the soil as root tips are sensitive to low O_2 conditions or changes in partitioning so more carbon is available for lateral roots. The second factor for the waterlogged treatment may be the lack of resources as water and nutrients become harder for roots to uptake in anaerobic conditions (Parent *et al.*, 2008). The depth of the troughs used was 20 cm with soil filled up to ~3 cm from the top of the trough. Plants in the 1x and 3x a week treatment were root bound as root length exceeded or equalled the depth of the soil which could have limited growth.

Watering frequency had no effect on taproot diameter but 'Monti' had a larger taproot diameter than 'Coolamon' (Figure 4.5). This may mean that 'Monti' had larger or more aerenchyma cells than 'Coolamon'. This would allow 'Monti' roots to obtain more oxygen from the atmosphere (Evans, 2004) and explain its higher growth in waterlogged conditions than 'Coolamon'. To confirm this root porosity measurements would need to be taken.

Plant population also decreased due to waterlogging. Plant population was not counted in the trough experiment but results from the dam experiment showed both subspecies had a reduction in plant number as flooding level increases (Figure 4.12). However, it appears that the reduction in ssp. *yanninicum* was less than that of ssp. *subterraneum*. Factors such as anaerobic soil conditions and reduction in photosynthesis probably contributed to a reduction in plant population, along with other factors such as root rot.

4.4.2 Petiole length and diameter

Waterlogging reduced petiole length by 50% in the trough experiment (Figure 4.7). This may reflect a decrease in nutrient uptake by the roots (Parent *et al.*, 2008) along with a reduction in photosynthesis. The reduced petiole length could also be due to reduced nitrogen fixation in the waterlogging treatment as nitrogen has an influence on plant height (Wilman and Asiegbu, 1982). No reduction in plant diameter was found in the dam experiment due to waterlogging (Table 4.7). Flooding treatments only lasted for three weeks in the dam experiment, compared with 7.5 weeks for the trough experiment, which may not have been sufficient time for changes to occur.

'Monti' had longer petioles than 'Coolamon' by ~4 cm (Figure 4.7). These results are consistent with the dam experiment where the two ssp. *yanninicum* cultivars had an average plant diameter of 18±0.42 cm compared with 15±0.42 cm for the two ssp. *subterraneum* cultivars. This increased petiole length gives 'Monti' an advantage in flooding situations as the leaves are more likely to be held out of the water than 'Coolamon'. 'Monti' also had a larger petiole diameter than 'Coolamon' by 0.40 mm (Figure 4.8). Watering frequency had no effect on petiole diameter suggesting that petiole diameter was genetically rather than environmentally controlled. The larger diameter is consistent with more structural capacity to support the longer petiole.

4.4.3 Nitrogen fixation

All treatments had a nodule colour score greater than 2 which suggests nitrogen fixation was occurring in all treatments (Table 4.4). Nodule size was the same for 'Monti' across all treatments. 'Coolamon' had smaller nodules than 'Monti' for the control and 1x a week treatment but the same for the 3x a week and waterlogged treatments. 'Coolamon' increased the size of the nodules when waterlogged or watered 3x a week. 'Coolamon' appeared to put energy into increasing nodule size but this still was insufficient to increase shoot DW under wet conditions. White clover has been found to increase the vacuole size in nodules when waterlogged to increase the amount of oxygen available to the *rhizobia* and therefore increase N_2 fixation (Pugh *et al.*, 1995). This could be a reason for the increased nodule size for 'Coolamon'.

4.4.4 Plant water relations

Osmotic potential was lower for the waterlogged subterranean clover than the other two treatments and the control at the first harvest (Figure 4.11). Osmotic potential has been shown to decrease due to waterlogging in other plant species such as castor bean after waterlogging for 15 days (Gadallah, 1995). The waterlogging in this experiment was for nearly eight weeks which may have given the subterranean clover plants time to adapt to the waterlogging in other ways instead of osmotic adjustment. The increase in osmotic potential between the waterlogged and control is only slight at 0.15 MPa and is likely insignificant.

RWC did not differ between the treatments or cultivars suggesting that the water status of the plants was similar. RWC averaged 90±2.34% for the first harvest which indicates all plants were well watered (Hsiao, 1990). This decreased to an average of 79±7.81% for this second harvest, 8 weeks after watering treatments finished, which may indicate the plants were slightly water stressed.

4.4.5 Photosynthesis

Photosynthetic rate decreased due to waterlogging (Table 4.5). This has been seen in other plant species such as wheat and ryegrass (Malik *et al.*, 2001; McFarlane *et al.*, 2003) but did not occur in white clover waterlogged for eight days (Blaikie *et al.*, 1988). The subterranean clover in this experiment was waterlogged for a longer period of 7.5 weeks so were likely more stressed than in the white clover experiment. The reduction in photosynthetic rate in waterlogged 'Coolamon' was probably due to a decrease in stomatal conductance. As the stomata close stomatal conductance reduces along with the amount of CO_2 absorbed which is necessary for photosynthesis. Stomatal closure usually occurs in drought stressed plants to reduce evaporation from the leaves (Taiz and Zeiger, 2006). However, waterlogged plants can also be water stressed due to decreased root conductance reducing water uptake, resulting in stomatal closure (Bradford and Hsiao, 1982). The RWC content of the waterlogged 'Coolamon' plants did not indicate that the plants were water stressed so it is unclear why stomatal closure occurred. However, this is consistent with their reduced shoot and root yields.

Waterlogging also reduced the photosynthetic rate for 'Monti', although not as much as 'Coolamon'. There was no change in stomatal conductance for 'Monti' that was waterlogged. The reduction in photosynthesis is likely due to leaf reddening as a response to waterlogging (Section 4.4.6). The production of anthocyanins, which cause leaf reddening, is associated with a reduction in photosynthetic rate as they reflect red light which is absorbed by chloroplasts (Chalker-Scott, 2002).

4.4.6 Leaf redness

'Monti' produced anthocyanins as a response to waterlogging. Waterlogged 'Monti' had the highest leaf redness score of 4±0.2 when watering treatments finished with all other treatments having a score of 1±0.2 (Table 4.6). 'Coolamon' had no change in leaf redness score due to waterlogging even though the plant was likely stressed and had large reductions in yield. Temporary anthocyanins are produced as a protective response. Anthocyanins in waterlogged plants help the leaf maintain water due to the increase in solutes. However in this experiment waterlogged 'Monti' did not have an increase in osmotic potential, suggesting that the anthocyanins may have another protective function. When exposed to cold temperatures, ssp. *yanninicum* had greater leaf redness than ssp. *subterraneum* (Teixeira et al., 2019). This, along with results from this experiment, could suggest that ssp. *yanninicum* had a greater ability to produce anthocyanins and therefore may be better at protecting itself against environmental stresses.

Eight weeks after treatments finished waterlogged 'Monti' still had the highest leaf redness score. However, this had reduced from the previous measurement to 2.75±0.09 suggesting that plants were no longer stressed and the temporary anthocyanins had begun to be metabolised.

4.5 Conclusion

'Monti' was more tolerant of waterlogging than 'Coolamon' as shown by a smaller reduction in yield when waterlogged. The morphological mechanism responsible for the waterlogging tolerance in 'Monti' was the increased production of lateral roots. Photosynthetic rate decreased under waterlogging for both cultivars but this rate was lower for 'Coolamon' due to increased stomatal closure. 'Monti' produced anthocyanins as a response to waterlogging that may provide an unknown protective function which could further contribute to the waterlogging tolerance of 'Monti'. From these results, it can be recommended to sow 'Monti' over 'Coolamon' in areas where winter waterlogging may occur.

5 GENERAL DISCUSSION AND CONCLUSIONS

5.1 Herbicides

The aim of Experiment 1 was to provide recommendations for herbicide use at establishment for subterranean clover cultivars in New Zealand. Both herbicides in Experiment 1, flumetsulam and imazethapyr, were effective at controlling broadleaf weeds, reducing broadleaf weed yield by ~1000 kg DM/ha for the season. Subterranean clover growth increased in all cultivars with both herbicide treatments due to reduced competition from broadleaf weeds. Flumetsulam has previously been reported by Gilmour (1996) to increase the re-emergence of subterranean clover the following autumn. In that study, subterranean clover populations increased from 150 seedlings/m^2 in the control to 240 seedlings/m^2. This was not the case in this experiment with all cultivars having a high re-emergence population of >490 seedlings/m^2 and no noticeable difference between herbicide treatments. Imazethapyr had greater residual control eight months after application. This suggests that subterranean clover may not need to be sprayed again the following year if imazethapyr is applied. However, longer term studies would be needed to confirm this. Flumetsulam had no residual control and would have to be applied to the emerging clover the following year to maintain a pure clover sward. Repeated applications over many years of ALS inhibiting herbicides are not recommended as broadleaf weeds can develop herbicide resistance to ALS inhibiting herbicides within five years (Zhou *et al.*, 2007). To prevent resistance alternating modes of action should be used. Subterranean clover is sensitive to most herbicide modes of action but Lewis (2017) identified a photosynthesis inhibitor, bentazone, as another potential mode of action for use on subterranean clovers. Further research into photosynthesis inhibitors use on subterranean clover cultivars could be explored as ALS inhibiting herbicides may not be a sustainable long term option.

5.2 Subterranean clover cultivars

'Antas' was one of the highest yielding cultivars in Experiment 1 with subterranean clover yield increasing in both herbicide treatments. This suggests either herbicide is suitable to use on this cultivar. 'Antas' was poorly managed, due to the need to graze in common, during the first grazing of this experiment and was grazed too low due to its upright growth habit. If managed properly ‘Antas’ yields may have exceeded those of ‘Napier’. This inability to manage cultivars differently highlights a limitation with this field experiment. ‘Antas’ had the lowest emergence in autumn 2019, after the simulated ‘false strike’, of 490 plants/m^2 which was not sufficient for a pure sward. However, cocksfoot could be over drilled to create a mixed pasture. Further research into ‘Antas’ grazing management is necessary to maximise its potential yield.

‘Napier’ was also a high yielding cultivar in Experiment 1 and was tolerant to both herbicides. This is likely due to ‘Napier’ being suited to the high rainfall year of the experiment as it is a ssp. *yanninicum.* During a dry year ‘Napier’ has been shown to have low yields (Lewis, 2017). However, ‘Napier’ is no longer available in New Zealand. Even with the high spring rainfall, the other two ssp. *yanninicum* cultivars, ‘Monti’ and ‘Trikkala’ performed poorly. All three ssp. *yanninicum* had seedling populations sufficient to establish a pure sward the following year. However, this may not be the case if there is a dry spring.

‘Coolamon’ growth was increased by flumetsulam at the first harvest but not imazethapyr. This shows cultivars reacted differently to herbicides and individual cultivar recommendations may be necessary. The three ssp. *subterraneum* cultivars did not differ in yield for the season. However, ‘Denmark’ had a lower proportion of white clover than the other two cultivars and also was very tolerant of grazing, suggesting it may be a better cultivar long term.

A potential difference in subspecies tolerance to herbicides was identified by Lewis (2017) with ssp. *subterraneum* cultivars being the most tolerant. This did not appear to be the case in this experiment as herbicide tolerance was based more on individual cultivars, rather than subspecies. For example, ‘Coolamon’ was tolerant to flumetsulam in the early stages whereas the other ssp. *subterraneum* cultivars ‘Denmark’ and ‘Narrikup’ were more sensitive.

Lucas *et al.* (2015) suggested sowing two subterranean clovers cultivars together. ‘Antas’ and ‘Denmark’ could be sown together, as ‘Antas’ is high yielding and ‘Denmark’ has been proven to be successful in long term studies with cocksfoot (Mills *et al.*, 2015). An application of imazethapyr at the 4+ leaf stage would provide broadleaf weed control and help establish a pure sward of clover. The following year cocksfoot could be over drilled to create a mixed pasture with high legume content. If the pasture was being sown in an area where waterlogging might occur 'Napier' or another ssp. *yanninicum*, such as 'Monti', could be used instead of 'Antas'.

There is also the potential to sow subterranean clover with plantain if using flumetsulam. Previous research has shown that plantain is initially suppressed by flumetsulam, which allows a strong establishment of clover, but recovers within 32 weeks (Gawn *et al.*, 2012).

Soft-seeded subterranean clover cultivars are successful in New Zealand conditions, as shown by the persistence of 'Mt Barker' (Lucas *et al.*, 2015). While cultivars such as 'Napier' and 'Coolamon' had good emergence in this experiment this may not be the case in a lower rainfall year. This experiment also highlights the problem of subterranean clover seed availability in New Zealand. 'Napier' is a high yielding cultivar that is likely to do well in wet conditions but is no longer available in New Zealand. 'Monti' the only ssp. *yanninicum* currently available is an early flowering cultivar which typically are not as successful as later flowering cultivars. There is a need for cultivars bred for New Zealand conditions with a reliable supply of seed to increase the use of subterranean clover by farmers especially as climate change progresses and droughts increase on the East Coast limiting the areas white clover can be grown.

5.3 Fertiliser requirements

Soil tests of Iversen 9 showed that sulphur levels were low (Table 3.2). The basal fertiliser should have been applied at sowing instead of when the clover began to show signs of nutrient deficiencies. There are difficulties in assessing plant available sulphur levels as the sulphate-S test is variable as sulphate is influenced by mineralisation (Rajendram *et al.*, 2008). The experiment was sown in late April after a month of higher than average rainfall (Section 3.2.4). This could be a reason why sulphur was low when soil tests were taken on 20 April 2018 as sulphate is prone to leaching. Increased levels of sulphur fertiliser may be required in areas with high rainfall.

5.4 White clover

The unusually high spring rainfall allowed the growth of white clover, which would not typically be the case in a dryland environment. This meant a high legume content was achieved even in the low yielding subterranean clover cultivars. White clover yield was higher in the low yielding cultivars and began to appear grow after the first grazing, as the sward was opened up. Although it is not necessarily a bad thing to have white clover growth in a subterranean clover pasture, it is likely to die as a result of water stress during the drier months. These results show that it is unnecessary to sow subterranean and white clover together if white clover seed is already present in the seed bank. If conditions are suitable for white clover it will germinate and grow which may reduce the yield of subterranean clover. However the total legume yield is likely to be the same.

White clover yield was reduced by imazethapyr at the third harvest. Therefore, flumetsulam may be a more appropriate herbicide to use on white clover.

5.5 Waterlogging

The aim of Experiment 2 was to investigate whether the *yanninicum* subspecies was a suitable subspecies to be used in winter wet conditions in New Zealand. From this experiment it can be concluded that 'Monti' was more tolerant of waterlogging than 'Coolamon' and is more suited to a winter wet environment due to its mechanism of increased root production and anthocyanin production. The waterlogging treatment in this experiment was extreme and lasted for eight weeks which would be uncommon on farms. However, the 3x a week treatment is more likely to occur. The yield of 'Coolamon' decreased under the 3x a week treatment suggesting that it was not suitable for use in winter wet conditions. It did recover by December but its yield would be reduced during the high growth period in early spring that coincides with the feed demand of lactating ewes (Brown *et al.*, 2006). 'Monti' is a more suitable choice as the yield increased with the 1x and 3x a week treatment compared with the control.

The yields of 'Monti' and 'Coolamon' were the same in the control treatments for the trough experiment for the first harvest. This is consistent with yields of 'Monti' and 'Coolamon' in the control plots in the herbicide experiment (Figure 3.5). However, at the second harvest for the trough experiment, ‘Monti’ had a higher yield the ‘Coolamon’ for the controls. This could be due to the cultivars growing together in the trough. ‘Monti’ had longer petioles which may have caused shading of ‘Coolamon’ as the clovers regrew after the harvest (Figure 4.7). The clovers were initially transplanted from the herbicide experiment and as such the ‘Coolamon’ plants were bigger and were likely to have been more tolerant of shading than post-harvest.

From the trough experiment it can only be determined there was a difference between these two cultivars, rather than subspecies. However, supporting evidence from the dam experiment and from the literature suggests that ssp. *yanninicum* is more tolerant of waterlogging than ssp. *subterraneum*. Further field experiments with a more cultivars from each subspecies should be conducted to provide more accurate recommendations.

5.6 Conclusions

- ALS inhibiting herbicides, imazethapyr and flumetsulam, when applied at the four-five trifoliate leaf stage increased subterranean clover yield and both resulted in similar reductions of broadleaf weeds. Imazethapyr had a longer residual and better weed control the following year but reduced early growth in all cultivars apart from ‘Antas’ and ‘Napier’.
- ‘Antas’ and ‘Napier’ are the highest yielding subterranean clover cultivars but further research needs to be done on ‘Antas’ management.
- ‘Monti’ was more tolerant of waterlogging than ‘Coolamon’ due to the main mechanism of increased lateral roots. However, ‘Monti’ yields were still reduced and neither cultivar could recover from severe waterlogging in eight weeks.

ACKNOWLEDGEMENTS

Firstly, I would like to acknowledge my supervisors Professor Derrick Moot and Professor Rainer Hofmann for their support and encouragement throughout the year.

Thank you to Roland Stead for providing the scholarship for this research.

Thank you to everyone at the Field Research centre. Dick Lucas for setting up and helping me run the experiment and answering all my questions about sub clover. Dave and Dan for helping set up the experiment and spraying the herbicides. Anna for giving me stats, Genstat, Sigmaplot and formatting advice. Keith for weather data and help with Greenseeker measurements. Malcolm for answering any questions I had and organising labour for harvest. Thank you to the casual staff that helped with data collection throughout the experiment.

Thank you to Stephen Stilwell for helping with photosynthesis measurements and plant water measurements.

Thank you to Richard Sedcole for statistical advice.

PLSC325 students for helping with data collection for the waterlogging experiment and my PLSC321 group for helping with the herbicide experiment.

Finally, thank you to my family for all the support throughout the last year. Especially my partner Callum, without your support I would not have been able to complete this thesis.

REFERENCES

Adami, M. F. F., Modolo, A. J., Adami, P. F., de Moraes, P. V., Erio, D., Pitta, C. S. R., Rossi, P. and Batista, V. V. 2017. White clover tolerance to herbicides applied at different rates and phenological stages. *African Journal of Agricultural Research*, **12**, 2336-2341.

Al-Khatib, K., Baumgartner, J. R., Peterson, D. E. and Currie, R. S. 1998. Imazethapyr resistance in common sunflower (*Helianthus annuus*). *Weed Science*, **46**, 403-407.

Armstrong, W., Justin, S., Beckett, P. and Lythe, S. 1991. Root adaptation to soil waterlogging. *Aquatic Botany*, **39**, 57-73.

BASF. 2016. Spinnaker® herbicide. Date Accessed:16/06/2019. Online: https://crop-solutions.basf.co.nz/files/_bSQy4t27iON9IgjV.pdf.

BASF. 2012. Spinnaker® WDG 700 herbicide. Date Accessed: 20/09/2019. Online: https://crop-solutions.basf.com.au/preview/f/product/59cb39de8d5c5b7640c83764/SPINNAKER®%20700%20WDG%20HERBICIDE%20Label%20ghs.pdf .

Blaikie, S., Martin, F., Mason, W. and Connor, D. 1988. Effects of soil water supply and temperature on the photosynthesis of white clover and paspalum in irrigated pastures. *Australian Journal of Experimental Agriculture*, **28**, 321-326.

Boutsalis, P. and Powles, S. 1995. Resistance of dicot weeds to acetolactate synthase (ALS)-inhibiting herbicides in Australia. *Weed Research*, **35**, 149-155.

Bowran, D. 1993. Differential response of four pasture legume species to broad-leaf herbicides. *In*: Proceedings of the 10th Australian Weeds Conference and 14th Asian Pacific Weed Science Society Conference, 6-10 September 1993, Brisbane, Australia. p 181-185.

Bradford, K. J. and Hsiao, T. C. 1982. Stomatal behavior and water relations of waterlogged tomato plants. *Plant Physiology*, **70**, 1508-1513.

Brown, H. E., Moot, D. J., Lucas, R. J. and Smith, M. 2006. Sub clover, cocksfoot and lucerne combine to improve dryland stock production. *Proceedings of the New Zealand Grassland Association*, **68**, 109-115.

Caradus, J. and Chapman, D. 1991. Variability of stolon characteristics and response to shading in two cultivars of white clover (*Trifolium repens* L.). *New Zealand Journal of Agricultural Research*, **34**, 239-247.

Caradus, J., Woodfield, D. and Stewart, A. 1995. Overview and vision for white clover. *In*. D. R. Woodfield (ed). White Clover: New Zealand's Competitive Edge. Proccedings of a New Zealand Grassland Association Inc Symposium held at Lincoln University, 21-22 November, 1995. Grassland Research and Practice Series No. **6**. 1-6.

Chalker-Scott, L. 2002. Do anthocyanins function as osmoregulators in leaf tissues? *Advances in Botanical Research*, **37**, 103-106.

Chalker-Scott, L. 1999. Environmental significance of anthocyanins in plant stress responses. *Photochemistry and Photobiology*, **70**, 1-9.

Chapman, D., Sheath, G., Macfarlane, M., Rumball, P., Cooper, B., Crouchley, G., Hoglund, J. and Widdup, K. 1986. Performance of subterranean and white clover varieties in dry hill country. *Proceedings of the New Zealand Grassland Association*, **47**, 53-62.

Cobb, A. H. and Reade, J. P. H. 2010. Herbicides and Plant Physiology (2nd Ed.). Hoboken, United Kingdom: Wiley-Blackwell. 286 pp.

Cocks, P. 1994. Colonization of a South Australian grassland by invading Mediterranean annual and perennial pasture species. *Australian Journal of Agricultural Research*, **45**, 1063-1076.

Cornforth, I. S. 1984. Fertiliser and lime recommendations for pastures and crops in New Zealand (2nd Ed). Wellington: Ministry of Agriculture & Fisheries. 76 pp.

Dear, B. and Sandral, G. 1999. The phytotoxicity of the herbicides bromoxynil, pyridate, imazethapyr and a bromoxynil+ diflufenican mixture on subterranean clover and lucerne seedlings. *Australian Journal of Experimental Agriculture*, **39**, 839-847.

Dear, B., Sandral, G. and Coombes, N. 1995. Differential tolerance of *Trifolium subterraneum* L.(subterranean clover) cultivars to broadleaf herbicides 1. Herbage yield. *Australian Journal of Experimental Agriculture*, **35**, 467-474.

Dear, B., Sandral, G. and Wilson, B. 2006. Tolerance of perennial pasture grass seedlings to pre-and post-emergent grass herbicides. *Australian Journal of Experimental Agriculture*, **46**, 637-644.

Dodd, M. B., Sheath, G. W. and Richardson, S. 1995. Development of subterranean clover (*Trifolium subterraneum* L.) genotypes for New Zealand pastures 1. Whatawhata persistence evaluation. *New Zealand Journal of Agricultural Research*, **38**, 33-47.

Ehlers, W. and Goss, M. 2003. Water Dynamics in Plant Production. Cambridge, United Kingdom: CABI. 396 pp.

Enloe, S. F., Johnson, J., Renz, M., Dorough, H. and Tucker, K. 2014. Hairy buttercup control and white clover tolerance to pasture herbicides. *Forage and Grazinglands*, **12**, 1-5.

Evans, D. E. 2004. Aerenchyma formation. *New Phytologist*, **161**, 35-49.

Evers, G. W., Grichar, W. J., Pohler, C. L. and Schubert, A. M. 1993. Tolerance of three annual forage legumes to selected postemergence herbicides. *Weed Technology*, **7**, 735-739.

Flett, S. P., Taylor, P. A. and Wakley, V. 1993. A method for the glasshouse evaluation of subterranean clover breeding lines for *Phytophthora clandestina* root rot. *In*: Proceedings of the XVII International Grassland Congress, 8-21 February 1993, Palmerston North, New Zealand. p 961-962.

Francis, C. and Devitt, A. 1969. The effect of waterlogging on the growth and isoflavone concentration of *Trifolium subterraneum* L. *Australian Journal of Agricultural Research*, **20**, 819-825.

Gadallah, M. 1995. Effect of waterlogging and kinetin on the stability of leaf membranes, leaf osmotic potential, soluble carbon and nitrogen compounds and chlorophyll content of *Ricinus* plants. *Phyton , International Journal of Experimental Botany*, **35**, 199-208.

Gawn, T., Harrington, K. and Matthew, C. 2012. Weed control in establishing mixed swards of clover plantain and chicory. *New Zealand Plant Protection*, **65**, 59-63.

Gibberd, M. and Cocks, P. 1997. Effect of waterlogging and soil pH on the micro-distribution of naturalised annual legumes. *Australian Journal of Agricultural Research*, **48**, 223-230.

Gibberd, M. R., Gray, J. D., Cocks, P. S. and Colmer, T. D. 2001. Waterlogging tolerance among a diverse range of *Trifolium* accessions is related to root porosity, lateral root formation and 'aerotropic rooting'. *Annals of Botany*, **88**, 579-589.

Gilmour, J. 1996. Broadstrike and Broadstrike+ diuron use in subterranean clover and medic pastures in Western Australia. *In*: Proceedings of the 11th Australian Weeds Conference, 30 September-3 October 1996, Melbourne, Australia. p 326-330.

Gimenez, A. E., York, A. C., Wilcut, J. W. and Batts, R. B. 1998. Annual grass control by glyphosate plus bentazon, chlorimuron, fomesafen, or imazethapyr mixtures. *Weed Technology*, **12**, 134-136.

Goetz, A. J., Lavy, T. L. and Gbur, E. E. 1990. Degradation and field persistence of imazethapyr. *Weed Science*, **38**, 421-428.

Grigg, D., Grigg, J. and Lucas, R. J. 2008. Maximising subterranean clover in Marlborough's hill country is key to weaning 80% of sale lambs prime. *Proceedings of the New Zealand Grassland Association*, **70**, 25-29.

Harris, B. and Husband, B. 1997. Flumetsulam for control of giant buttercup in pasture. *In*: Proceedings of the New Zealand Plant Protection Conference, 18-21 August 1997, Lincoln University. p 472-476.

Harvey, I. C. and Harvey, B. M. 2009. Pasture diseases in New Zealand. Christchurch, New Zealand: The Caxton Press. 144 pp.

Hollaway, K., Kookana, R. S., Noy, D., Smith, J. and Wilhelm, N. 2006a. Crop damage caused by residual acetolactate synthase herbicides in the soils of south-eastern Australia. *Australian Journal of Experimental Agriculture*, **46**, 1323-1331.

Hollaway, K., Kookana, R. S., Noy, D., Smith, J. and Wilhelm, N. 2006b. Persistence and leaching of imazethapyr and flumetsulam herbicides over a 4-year period in the highly alkaline soils of south-eastern Australia. *Australian Journal of Experimental Agriculture*, **46**, 669-674.

Hsiao, T. H. 1990. Measurements of Plant Water Status. *In:* B. A. Stewart and D. R. Nielsen, (eds). Irrigation of Agricultural Crops. Wiscosnsin: American Society of Agronomy, Inc. p 243-279.

James, E., Minchin, F. and Sprent, J. 1992. The physiology and nitrogen-fixing capability of aquatically and terrestrially grown *Neptunia plena*: The importance of nodule oxygen supply. *Annals of Botany*, **69**, 181-187.

Jones, C. A. and Kiniry, J. R. 1986. CERES-Maize : a simulation model of maize growth and development. College Station: Texas A&M University Press. 194 pp.

Katznelson, J. 1970. Edaphic factors in the distribution of subterranean clover in the Mediterranean region. *In*: Proceedings of the XI International Grassland Congress 13-23 April 1970, Surfers Paradise, Queensland, Australia. p 192-196.

Knowles, I., Fraser, T. and Daly, M. 2003. White clover: Loss in drought and subsequent recovery. *In:* D. J. Moot (ed). Legumes for Dryland Pastures. Proceedings of a New Zealand Grassland Association Inc Symposium held at Lincoln University, 18-19 November, 2003: Grassland Research and Practice Series No. **11**, 37-41

Ladner, D. W. 1990. Structure-activity relationships among the imidazolinone herbicides. *Pesticide Science*, **29**, 317-333.

Lewis, T. R. 2017. Yield and phytotoxicity responses of subterranean clover (*Trifolium subterraneum* L.) sprayed with different herbicides for broadleaf weed control. Masters thesis, Lincoln University, Canterbury, New Zealand. 155 pp. Online: https://hdl.handle.net/10182/8934.

Lonza. 2018. Headstart® herbicide. Date Accessed:15/06/2019. https://lonza.co.nz/wp-content/uploads/2018/01/Headstart®-label.pdf.

Lucas, R. J., Mills, A., Wright, S., Black, A. and Moot, D. J. 2015. Selection of sub clover cultivars for New Zealand dryland pastures. *Journal of New Zealand Grasslands*, **77**, 203-210.

Lucas, R. J., Smith, M. C., Jarvis, P., Mills, A. and Moot, D. J. 2010. Nitrogen fixation by subterranean and white clovers in dryland cocksfoot pastures. *Proceedings of the New Zealand Grassland Association*, **72**, 141-146.

Malik, A. I., Colmer, T. D., Lambers, H. and Schortemeyer, M. 2001. Changes in physiological and morphological traits of roots and shoots of wheat in response to different depths of waterlogging. *Functional Plant Biology*, **28**, 1121-1131.

Mallory-Smith, C. A., Thill, D. C. and Dial, M. J. 1990. Identification of sulfonylurea herbicide-resistant prickly lettuce (*Lactuca serriola*). *Weed Technology*, **4**, 163-168.

McCurdy, J. D., McElroy, J. S. and Flessner, M. L. 2013. Differential response of four *Trifolium* species to common broadleaf herbicides: implications for mixed grass-legume swards. *Weed Technology*, **27**, 123-128.

McDowell, R., Condron, L., Main, B. and Dastgheib, F. 1997. Dissipation of imazapyr, flumetsulam and thifensulfuron in soil. *Weed Research*, **37**, 381-389.

McDowell, R., Dastgheib, F. and Condron, L. 1996. Persistence of acetolactate synthase inhibiting herbicides in a Canterbury soil. *In*: Proceedings of the New Zealand Plant Protection Conference, 13-15 August 1996, Nelson, New Zealand. p 198-201.

McFarlane, N., Ciavarella, T. and Smith, K. F. 2003. The effects of waterlogging on growth, photosynthesis and biomass allocation in perennial ryegrass (*Lolium perenne* L.) genotypes with contrasting root development. *The Journal of Agricultural Science*, **141**, 241-248.

Mills, A. 2007. Understanding constraints to cocksfoot (*Dactylis glomerata* L.) based pasture production. Ph.D thesis, Lincoln University, Canterbury, New Zealand. 224 pp. Online: https://hdl.handle.net/10182/32.

Mills, A., Lucas, R. and Moot, D. 2015. 'MaxClover'grazing experiment: I. Annual yields, botanical composition and growth rates of six dryland pastures over nine years. *Grass and Forage Science*, **70**, 557-570.

Mills, A., Smith, M., Lucas, R. J. and Moot, D. J. 2008. Dryland pasture yields and botanical composition over 5 years under sheep grazing in Canterbury. *Proceedings of the New Zealand Grassland Association*, **70**, 37-44.

Monk, S., Moot, D., Belgrave, B., Rolston, M. and Caradus, J. 2016. Availability of seed for hill country adapted forage legumes *In:* E. R. Thom (ed). Hill Country Symposium. Proceedings of a New Zealand Grassland Association Inc Symposium held in Rotorua, 12-13 April, 2016. Grassland Research and Practice Series No. **16**. 257-267

Monks, D. P. 2009. The vegetative and reproductive development of balansa clover. Ph.D thesis, Lincoln University, Canterbury, New Zealand. Online: https://hdl.handle.net/10182/1337.

Moot, D., Black, A., Scott, W. and Richardon, J. 2003. Leaf development and dry matter production of subterranean clover cultivars in relation to autumn sward management. *In:* D. J. Moot (ed). Legumes for Dryland Pastures. Proceedings of a New Zealand Grassland Association Inc Symposium held at Lincoln University, 18-19 November, 2003. Grassland Research and Practice Series No. **11**. 193-200.

Moot, D., Scott, W., Roy, A. and Nicholls, A. 2000. Base temperature and thermal time requirements for germination and emergence of temperate pasture species. *New Zealand Journal of Agricultural Research*, **43**, 15-25.

Nichols, P., Foster, K., Piano, E., Pecetti, L., Kaur, P., Ghamkhar, K. and Collins, W. 2013a. Genetic improvement of subterranean clover (*Trifolium subterraneum* L.). 1. Germplasm, traits and future prospects. *Crop and Pasture Science*, **64**, 312-346.

Nichols, P., Revell, C., Humphries, A., Howie, J., Hall, E., Sandral, G., Ghamkhar, K. and Harris, C. 2013b. Temperate pasture legumes in Australia—their history, current use, and future prospects. *Crop and Pasture Science*, **63**, 691-725.

Nori, H. A. 2013. The development and growth of annual clovers for use in dryland pasture. Ph.D thesis, Lincoln University, Canterbury, New Zealand. 298 pp. Online: https://hdl.handle.net/10182/5634.

Novachem. 2019. New Zealand Novachem Agrichemical Manual. Date Accessed: 20/09/2019. Online: https://www.novachem.co.nz/.

Oliveira, J. S. 2015. Growth and development of potato (*Solanum tuberosum* L.) crops after different cool season storage. Ph.D Thesis, Lincoln University, Canterbury, New Zealand. 321 pp. Online: https://hdl.handle.net/10182/6494.

Olykan, S. T., Lucas, R. J. and Moot, D. J. 2019. Sampling methods for clover species in grazed pastures to diagnose mineral deficiencies. *Journal of New Zealand Grasslands*, **81**, 261-267.

Parent, C., Capelli, N., Berger, A., Crèvecoeur, M. and Dat, J. F. 2008. An overview of plant responses to soil waterlogging. *Plant Stress*, **2**, 20-27.

Peoples, M. B., Faizah, A., Rerkasem, B. and Herridge, D. F. 1989. Methods for evaluating nitrogen fixation by nodulated legumes in the field. ACIR Monograph, **No.** 11. pp 76.

PubChem. 2005a. Imazethapyr Date Accessed:21/09/2018. https://pubchem.ncbi.nlm.nih.gov/compound/Imazethapyr. Last Updated: 26/10/2019.

PubChem. 2005b. Flumetsulam Date Accessed:21/09/2019. https://pubchem.ncbi.nlm.nih.gov/compound/Flumetsulam. Last Updated: 14/09/2019.

Pugh, R., Witty, J. F., Mytton, L. R. and Minchin, F. R. 1995. The effect of waterlogging on nitrogen fixation and nodule morphology in soil-grown white clover (*Trifolium repens* L.). *Journal of Experimental Botany*, **46**, 285-290.

Rajendram, G., Ghani, A., Waller, J., Kear, M., Watkinson, J., Benge, K. and Wheeler, D. 2008. Total Sulphur: A better predictor of sulphur deficiency in pastoral soils. *In:* L. D. Currie and L. J. Yates (eds). Carbon and nutrient management in agriculture. Occasional Report No. 21. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand.

Reuter, D. J. and Robinson, J. B. 1997. Plant analysis: An interpretation manual (2nd Ed). Australia: CSRIO Publishing. 585 pp.

Rogers, M. and West, D. 1993. The effects of rootzone salinity and hypoxia on shoot and root growth in *Trifolium* species. *Annals of Botany*, **72**, 503-509.

Sandral, G. and Dear, B. 2005. Weed control options in annual pasture legumes. Rural Industries Research and Development Corporation. Barton, ACT. 22 pp.

Saxby, S. H. 1956. The history of subterranean clover in New Zealand. *New Zealand Journal of Agriculture*, **92**, 521-526.

Seng, K. H. 2014. The effects of drought, waterlogging and heat stress on tomatoes (*Solanum lycopersicon* L.). Ph.D thesis, Lincoln University. 289 pp. Online: https://hdl.handle.net/10182/6432.

Shaw, D. R. and Murphy, G. P. 1997. Field persistence of bioavailable flumetsulam. *Weed Science*, **45**, 568-572.

Singh, B. K. and Shaner, D. L. 1995. Biosynthesis of branched chain amino acids: From test tube to field. *The Plant Cell*, **7**, 935-944.

Smetham, M. and Ying, C. W. 1991. Establishment of subterranean clover (*Trifolium subterraneum* L.) in New Zealand 1. Hardseededness and autumn germination. *New Zealand Journal of Agricultural Research*, **34**, 31-44.

Smetham, M. L. 2003. A review of subterranean clover (*Trifolium subterraneum* L.): its ecology, and use as a pasture legume in Australasia. *Advances in Agronomy*, **79**, 303-350.

Sokal, R. R. and Rohlf, F. J. 1981. Biometry: the principles and practice of statistics in biological research (2nd Ed.). New York: W.H. Freeman. 859 pp.

Suckling, F., Forde, M. and Williams, W. 1983. Naturalised subterranean clover in New Zealand. *New Zealand Journal of Agricultural Research*, **26**, 35-43.

Taiz, L. and Zeiger, E. 2006. Plant Physiology (4th Ed). Sunderland, Massachusetts: Sinauer Associates, Inc. 764 pp.

Teixeira, C. S., Lucas, R. J. and Moot, D. J. 2018. Seed yield and subsequent emergence pattern of subterranean clover cultivars in response to summer rain. *New Zealand Grassland Association*, **80**, 91-96.

Teixeira, C. S. P., Lucas, R. J., Olykan, S. T. and Moot, D. J. 2019. Cause of leaf reddening in subterranean clover cultivars. *New Zealand Journal of Agricultural Research*. In press.

Thomas, H. 1986. Effect of Rate of Dehydration on Leaf Water Status and Osmotic Adjustment in *Dactylis glomerate* L., *Lolium perenne* L. and *L. multiflorum* Lam. *Annals of Botany*, 225-235.

Tonmukayakul, N. 2009. Water use efficiency of six dryland pastures in Canterbury. Masters thesis, Lincoln University, Canterbury, New Zealand. 85 pp. Online: https://hdl.handle.net/10182/1498.

Tranel, P. J. and Wright, T. R. 2002. Resistance of weeds to ALS-inhibiting herbicides: what have we learned? *Weed Science*, **50**, 700-712.

Whitcomb, C. E. 1999. An introduction to ALS-inhibiting herbicides. *Toxicology and Industrial Health*, **15**, 232-240.

Wilman, D. and Asiegbu, J. 1982. The effects of clover variety, cutting interval and nitrogen application on herbage yields, proportions and heights in perennial ryegrass-white clover swards. *Grass and Forage Science*, **37**, 1-13.

Wong, D., D'antuono, M., Barbetti, M. and Sivasithamparam, K. 1986. Inter-relationship between shoot weight, severity of root rot and survival rate of subterranean clover inoculated with certain pathogenic fungi. *Plant and Soil*, **96**, 141-143.

Zhou, Q., Liu, W., Zhang, Y. and Liu, K. K. 2007. Action mechanisms of acetolactate synthase-inhibiting herbicides. *Pesticide Biochemistry and Physiology*, **89**, 89-96.

APPENDICES

Appendix 1 Outline of Experiment 1.

April 2018	
20 April 2018	Experiment 1 sown
May	
1 May 2018	Subterranean clover emergence
June	
13 June 2018	Seedling population counted
21 June 2018	Seedling population counted
July	
4 July 2018	Herbicide treatments applied and seedling population counted
19 July 2018	Seedling population counted
August	
24 August 2018	Yellowing of sub clover leaves noticed, foliar samples taken for nutrient analysis
September	
4 September 2018	Plots harvested
11 September 2018	Plots fertilised
October	
3 October 2018	Plots harvested
November	
4 November 2018	Plots harvested
December	
6 December 2018	Plots harvested
February 2019	
21 February 2019	Subterranean clover emergence counted and plots sprayed with 'Buster'.
March	
15 March 2019	Subterranean clover emergence counted and docks scored

Appendix 2 Photo examples of subterranean clover emergence scale used in Experiment 1 at Iversen 9, Lincoln University, Canterbury, New Zealand.

Appendix 3 Total dry matter yield of subterranean clover cultivars 1 May 2018 – 6 December 2018 after treatment with herbicides at establishment, at Iversen 9, Lincoln University, Canterbury, New Zealand.

Cultivar	Control	Flumetsulam	Imazethapyr	Average
Total DM				
Antas	7280	8800	8400	8160_{a}
Coolamon	6330	6850	5720	6300_{b}
Denmark	6530	6690	6420	6550_{b}
Monti	6720	7310	6620	6880_{b}
Napier	8620	8280	8150	8350_{a}
Narrikup	6520	7280	6620	6800_{b}
Trikkala	6890	6340	5550	6260_{b}
P value – HB	0.208	SEM – HB	206.4	
P value – CV	0.005	SEM – CV	397.9	
P value – HB*CV	0.432	SEM – HB*CV	546.4	
Subterranean clover DM				
Antas	4730	7030	7340	6370_{a}
Coolamon	3450	5210	4620	4430_{bc}
Denmark	4400	5330	5200	4970_{b}
Monti	3520	5280	3940	4250_{bc}
Napier	6910	7050	7210	7060_{a}
Narrikup	2960	4800	4270	4010_{bc}
Trikkala	3400	4380	4170	3980_{c}
Average	4200_{b}	5580_{a}	5250_{a}	
P value – HB	0.003	SEM – HB	172.8	
P value – CV	<0.001	SEM – CV	328.5	
P value – HB*CV	0.642	SEM – HB*CV	542.8	
White clover DM				
Antas	731	766	472	656_{bc}
Coolamon	888	641	630	719_{bc}
Denmark	754	618	638	670_{bc}
Monti	1070	1180	1170	1140_{ab}
Napier	355	312	443	370_{c}
Narrikup	1620	927	1400	1320_{a}
Trikkala	1370	1340	515	1070_{ab}
P value – HB	0.432	SEM – HB	111.9	
P value – CV	0.024	SEM – CV	185.3	

P value – HB*CV	0.240	SEM – HB*CV	262.1
Cultivar	Control	Flumetsulam	Imazethapyr
Broadleaf weed DM			
Antas	1350	472	322
Coolamon	1600	488	184
Denmark	1200	368	408
Monti	1820	428	1150
Napier	1080	480	211
Narrikup	1610	927	596
Trikkala	1840	231	580
Average	1500_a	484_b	492_b
P value – HB	<0.001	SEM – HB	100.3
P value – CV	0.217	SEM – CV	162.2
P value – HB*CV	0.142	SEM – HB*CV	237.7
Grass weed DM			
Antas	325	194	60.0
Coolamon	105	374	92.4
Denmark	54.8	180	52.5
Monti	84.6	233	142
Napier	89.0	180	55.2
Narrikup	93.7	300	64.6
Trikkala	104	201	74.5
P value – HB	0.082	SEM – HB	41.82
P value – CV	0.652	SEM – CV	45.33
P value – HB*CV	0.499	SEM – HB*CV	81.31
Dead material DM			
Antas	150	345	204
Coolamon	290	143	189
Denmark	123	198	121
Monti	221	194	217
Napier	181	260	228
Narrikup	238	327	300
Trikkala	186	194	215
P value – HB	0.465	SEM – HB	21.36
P value – CV	0.754	SEM – CV	56.37
P value – HB*CV	0.791	SEM – HB*CV	78.77

Appendix 4 Dry matter yield of subterranean clover cultivars on 3 October 2018 after treatment with herbicides at establishment, at Iversen 9, Lincoln University, Canterbury, New Zealand.

Cultivar	Control	Flumetsulam	Imazethapyr
Total DM			
Antas	2190_{bcde}	2830_{a}	2550_{abc}
Coolamon	1900_{bcde}	2180_{bcde}	1230_{fg}
Denmark	1950_{bcde}	1280_{ef}	952_{g}
Monti	1950_{bcde}	2050_{bcde}	1550_{def}
Napier	2230_{abcd}	2630_{ab}	2460_{abc}
Narrikup	1730_{bcde}	1710_{cdef}	1420_{def}
Trikkala	2070_{bcde}	1700_{cdef}	1510_{def}
P value – HB	0.141	SEM – HB	126.5
P value – CV	0.078	SEM – CV	275.4
P value – HB*CV	0.005	SEM – HB*CV	326.5
Subterranean clover DM			
Antas	1460_{defg}	2610_{a}	2450_{ab}
Coolamon	1080_{fg}	1840_{bcde}	1140_{fg}
Denmark	1100_{fg}	1130_{fg}	834_{g}
Monti	1230_{efg}	1750_{cdef}	1290_{efg}
Napier	1390_{efg}	2120_{abc}	2110_{abcd}
Narrikup	971_{g}	1120_{fg}	803_{g}
Trikkala	1080_{g}	1390_{efg}	1230_{efg}
P value – HB	0.014	SEM – HB	86.10
P value – CV	0.001	SEM – CV	181.3
P value – HB*CV	0.010	SEM – HB*CV	231.5
White clover DM			
Antas	36.9	21.6	11.2
Coolamon	45.5	111	37.7
Denmark	86.7	42.5	38.5
Monti	74.8	94.0	94.3
Napier	58.0	96.6	177
Narrikup	226	252	481
Trikkala	124	182	94.7
P value – HB	0.676	SEM – HB	31.21
P value – CV	0.593	SEM – CV	110.2
P value – HB*CV	0.701	SEM – HB*CV	126.8

Broadleaf weed DM			
Antas	460	117	61.2
Coolamon	711	104	29.3
Denmark	717	67.6	52.4
Monti	623	135	129
Napier	729	286	140
Narrikup	501	234	102
Trikkala	834	60.5	166
Average	653_a	143_b	97.0_b

P value – HB	<0.001	SEM – HB	55.01
P value – CV	0.807	SEM – CV	80.44
P value – HB*CV	0.566	SEM – HB*CV	47.48

Grass weed DM			
Antas	240	56.4	19.8
Coolamon	39.6	127	28.5
Denmark	33.6	40.5	26.9
Monti	17.3	71.7	36.8
Napier	29.7	106	36.8
Narrikup	30.2	103	30.7
Trikkala	32.1	68.3	21.3

P value – HB	0.345	SEM – HB	23.71
P value – CV	0.632	SEM – CV	28.13
P value – HB*CV	0.324	SEM – HB*CV	50.86

Dead material DM			
Antas	1.25	25.4	4.75
Coolamon	30.3	0.00	0.00
Denmark	13.0	0.76	0.80
Monti	7.24	1.10	0.38
Napier	17.0	17.7	0.00
Narrikup	3.38	0.00	2.93
Trikkala	4.92	1.23	0.00

P value – HB	0.206	SEM – HB	3.38
P value – CV	0.544	SEM – CV	4.59
P value – HB*CV	0.556	SEM – HB*CV	8.91

Appendix 5 Dry matter yield of subterranean clover cultivars on 2 November 2018 after treatment with herbicides at establishment, at Iversen 9, Lincoln University, Canterbury, New Zealand.

Cultivar	Control	Flumetsulam	Spinnaker®	Average
Total DM				
Antas	1290	1420	1370	1360_{c}
Coolamon	1350	2190	1330	1640_{ab}
Denmark	1280	1960	1700	1640_{ab}
Monti	1550	1790	1760	1700_{ab}
Napier	1580	2290	1530	1800_{ab}
Narrikup	1590	2290	1690	1860_{a}
Trikkala	1660	1750	1280	1560_{bc}
Average	1470_{b}	1960_{a}	1520_{b}	
P value – HB	0.016	SEM – HB	89.31	
P value – CV	0.008	SEM – CV	80.34	
P value – HB*CV	0.136	SEM – HB*CV	170.3	
Subterranean clover DM				
Antas	811	895	1040	916_{d}
Coolamon	911	1740	1180	1280_{abc}
Denmark	946	1640	1460	1350_{abc}
Monti	826	1180	1260	1090_{cd}
Napier	1160	2020	1400	1530_{a}
Narrikup	992	1690	1430	1370_{ab}
Trikkala	1080	1310	1090	1160_{bcd}
Average	960_{c}	1500_{a}	1270_{b}	
P value – HB	0.006	SEM – HB	74.21	
P value – CV	0.004	SEM – CV	92.04	
P value – HB*CV	0.455	SEM – HB*CV	178.7	
White clover DM				
Antas	150	217	70.4	142_{ab}
Coolamon	112	76.6	31.6	73.4_{c}
Denmark	97.3	111	114	108_{bc}
Monti	219	226	156	200_{a}
Napier	84.3	80.8	35.7	66.9_{c}
Narrikup	206	118	104	142_{ab}
Trikkala	120	217	47.7	128_{bc}
Average	141_{a}	150_{a}	79.8_{b}	

P value – HB	0.032	SEM – HB	15.06
P value – CV	0.007	SEM – CV	21.97
P value – HB*CV	0.491	SEM – HB*CV	39.51
Cultivar	Control	Flumetsulam	Spinnaker®
Broadleaf weed DM			
Antas	233	129	137
Coolamon	261	140	42.9
Denmark	215	27.8	77.9
Monti	351	245	221
Napier	244	87.6	62.2
Narrikup	308	140	101
Trikkala	346	89.3	93.3
Average	280_a	123_b	105_b
P value – HB	0.008	SEM – HB	27.16
P value – CV	0.095	SEM – CV	35.83
P value – HB*CV	0.971	SEM – HB*CV	63.71
Grass weed DM			
Antas	81.2	134	33.4
Coolamon	43.7	201	54.1
Denmark	14.2	126	25.7
Monti	63.9	89.8	69.3
Napier	53.8	73.6	18.4
Narrikup	35.3	179	30.6
Trikkala	30.3	122	34.9
Average	46.1_b	132_a	38.1_b
P value – HB	0.036	SEM – HB	21.17
P value – CV	0.825	SEM – CV	26.04
P value – HB*CV	0.877	SEM – HB*CV	45.40
Dead material DM			
Antas	12.8	44.6	86.9
Coolamon	22.6	32.4	23.3
Denmark	5.26	49.0	20.7
Monti	85.1	49.3	52.7
Napier	44.5	28.8	19.7
Narrikup	48.9	172	29.6
Trikkala	79.7	15.8	17.8
Average	46.1_b	132_a	38.1_b

P value – HB	0.036	SEM – HB	21.17
P value – CV	0.825	SEM – CV	26.04
P value – HB*CV	0.877	SEM – HB*CV	45.40

Appendix 6 Dry matter yield of subterranean clover cultivars on 6 December 2018 after treatment with herbicides at establishment, at Iversen 9, Lincoln University, Canterbury, New Zealand.

Cultivar	Control	Flumetsulam	Spinnaker®	Average
Total DM				
Antas	3800	4550	4480	4280_{a}
Coolamon	3080	2480	3150	2900_{c}
Denmark	3310	3450	3770	3510_{bc}
Monti	3220	3470	3310	3330_{c}
Napier	4810	3360	4160	4110_{ab}
Narrikup	3200	3270	3510	3330_{c}
Trikkala	3160	2890	2760	2940_{c}
P value – HB	0.474	SEM – HB	130.4	
P value – CV	0.002	SEM – CV	223.5	
P value – HB*CV	0.418	SEM – HB*CV	372.3	
Subterranean clover DM				
Antas	2460	3520	3850	3280_{ab}
Coolamon	1460	1630	2300	1800_{c}
Denmark	2360	2550	2900	2600_{b}
Monti	1470	2350	1390	1740_{c}
Napier	4360	2900	3710	3660_{a}
Narrikup	1000	1990	2030	1670_{c}
Trikkala	1240	1680	1850	1590_{c}
Average	2050_{b}	2380_{a}	2577_{a}	
P value – HB	0.026	SEM – HB	99.44	
P value – CV	<0.001	SEM – CV	240.9	
P value – HB*CV	0.259	SEM – HB*CV	415.3	
White clover DM				
Antas	543	527	390	487_{ab}
Coolamon	730	453	561	581_{ab}
Denmark	570	464	486	507_{ab}
Monti	773	859	921	851_{a}
Napier	213	135	230	192_{b}
Narrikup	1190	558	813	855_{a}
Trikkala	1120	937	373	810_{a}
P value – HB	0.322	SEM – HB	91.03	
P value – CV	0.031	SEM – CV	140.5	

P value – HB*CV	0.449	SEM – HB*CV	214.6

Cultivar	Control	Flumetsulam	Spinnaker®
Broadleaf weed DM			
Antas	653_{abcd}	226_{ef}	123_{f}
Coolamon	629_{abcd}	244_{ef}	112_{f}
Denmark	267_{def}	272_{def}	277_{def}
Monti	849_{a}	48.5_{f}	797_{ab}
Napier	110_{f}	106_{f}	8.40_{f}
Narrikup	797_{abc}	554_{abcde}	394_{bdef}
Trikkala	660_{abcd}	81.4	321_{def}
P value – HB	0.001	SEM – HB	37.21
P value – CV	0.030	SEM – CV	99.53
P value – HB*CV	0.015	SEM – HB*CV	140.4
Grass weed DM			
Antas	3.81_{e}	4.43_{e}	6.84_{de}
Coolamon	22.0_{bcde}	45.5_{ab}	9.83_{de}
Denmark	6.97_{de}	13.6_{cde}	0.00_{e}
Monti	3.35_{e}	71.6_{a}	36.0_{bcd}
Napier	5.49_{e}	0.00_{e}	0.00_{e}
Narrikup	28.1_{bcde}	17.7_{bcde}	3.23_{e}
Trikkala	41.7_{bc}	11.4_{de}	18.2_{bcde}
P value – HB	0.083	SEM – HB	3.291
P value – CV	0.004	SEM – CV	5.817
P value – HB*CV	0.0.17	SEM – HB*CV	10.16
Dead material DM			
Antas	136	275	113
Coolamon	237	110	166
Denmark	105	148	100
Monti	129	144	164
Napier	119	214	208
Narrikup	186	155	267
Trikkala	101	177	197
P value – HB	0.449	SEM – HB	17.74
P value – CV	0.931	SEM – CV	50.13
P value – HB*CV	0.626	SEM – HB*CV	69.98

Appendix 7 Botanical composition (%) of subterranean clover cultivars on 3 October 2018 after treatment with herbicides at establishment, at Iversen 9, Lincoln University, Canterbury, New Zealand.

Cultivar	Control	Flumetsulam	Spinnaker®	Average
Subterranean clover (%)				
Antas	68.7	91.2	96.4	85.5_{a}
Coolamon	58.7	85.1	92.4	78.7_{ab}
Denmark	57.6	87.9	87.3	77.6_{ab}
Monti	61.8	82.8	83.6	76.1_{ab}
Napier	64.4	81.5	87.4	77.7_{ab}
Narrikup	58.7	67.6	60.6	62.3_{c}
Trikkala	53.6	82.1	83.2	73.0_{bc}
Average	60.5_{b}	82.6_{a}	84.4_{a}	

P value – HB	<0.001	SEM – HB	1.061,1.047*
P value – CV	0.032	SEM – CV	2.203,2.125*
P value – HB*CV	0.196	SEM – HB*CV	3.033,2.896*

Cultivar	Control	Flumetsulam	Spinnaker®
White clover (%)			
Antas	1.82	1.03	0.45
Coolamon	2.37	4.83	3.17
Denmark	4.63	3.63	3.99
Monti	4.57	6.00	5.84
Napier	2.42	4.14	6.12
Narrikup	9.84	12.6	20.8
Trikkala	6.00	9.28	5.42

P value – HB	0.876	SEM – HB	1.154,1.335*
P value – CV	0.245	SEM – CV	0.404,0.427*
P value – HB*CV	0.864	SEM – HB*CV	1.341,1.603*

Cultivar	Control	Flumetsulam	Spinnaker®
Broadleaf weed (%)			
Antas	19.0	4.82	2.20
Coolamon	36.1	4.83	2.41
Denmark	35.5	5.32	5.60
Monti	32.4	7.63	7.89
Napier	31.4	10.1	5.20
Narrikup	29.2	12.6	13.8
Trikkala	38.5	3.30	9.33

Average	31.7_a	6.95_b	6.63_b
P value – HB	<0.001	SEM – HB	6.302,8.246*
P value – CV	0.206	SEM – CV	1.452,1.536*
P value – HB*CV	0.226	SEM – HB*CV	1.985,2.145*
Grass weed (%)			
Antas	10.4	2.21	0.78
Coolamon	1.69	5.27	2.04
Denmark	1.68	3.09	2.99
Monti	0.95	3.47	2.66
Napier	1.23	3.75	1.32
Narrikup	2.09	7.17	4.78
Trikkala	1.68	5.23	2.05
P value – HB	0.466	SEM – HB	1.088
P value – CV	0.847	SEM – CV	1.537
P value – HB*CV	0.238	SEM – HB*CV	2.454
Dead material (%)			
Antas	0.06	0.69	0.17
Coolamon	1.15	0.00	0.00
Denmark	0.57	0.08	0.09
Monti	0.28	0.08	0.03
Napier	0.56	0.54	0.00
Narrikup	0.17	0.00	0.11
Trikkala	0.24	0.06	0.00
P value – HB	0.173	SEM – HB	0.1226
P value – CV	0.652	SEM – CV	0.1498
P value – HB*CV	0.492	SEM – HB*CV	0.2915

Note - * indicates that the data was arcsine transformed and the SEM of mean had been back-transformed.

Appendix 8 Botanical composition (%) of subterranean clover cultivars on 2 November 2018 after treatment with herbicides at establishment, at Iversen 9, Lincoln University, Canterbury, New Zealand.

Cultivar	Control	Flumetsulam	Spinnaker®	Average
Subterranean clover (%)				
Antas	66.6	63.8	76.7	69.0_{c}
Coolamon	67.8	79.3	89.3	78.8_{ab}
Denmark	72.9	82.6	86.3	80.6_{ab}
Monti	54.0	63.7	71.1	62.9_{c}
Napier	71.9	87.8	90.4	83.4_{a}
Narrikup	61.8	72.0	83.6	72.4_{b}
Trikkala	66.0	74.1	85.6	75.2_{abc}
Average	65.9_{c}	74.8_{b}	83.3_{a}	

P value – HB	0.009	SEM – HB	0.9452,1.688*
P value – CV	0.003	SEM – CV	1.295,1.859*
P value – HB*CV	0.924	SEM – HB*CV	2.951,2.833*

Cultivar	Control	Flumetsulam	Spinnaker®	Average
White clover (%)				
Antas	11.5	14.7	5.29	10.5_{ab}
Coolamon	7.90	3.82	2.19	4.63_{d}
Denmark	7.61	5.92	6.56	6.70_{cd}
Monti	14.0	13.1	9.32	12.1_{a}
Napier	5.47	3.73	2.63	3.94_{d}
Narrikup	13.4	5.67	6.54	8.53_{abc}
Trikkala	7.20	11.9	3.64	7.57_{bcd}
Average	9.56_{a}	8.39_{a}	5.17_{b}	

P value – HB	0.011	SEM – HB	0.6975
P value – CV	0.002	SEM – CV	1.225
P value – HB*CV	0.383	SEM – HB*CV	2.243

Cultivar	Control	Flumetsulam	Spinnaker®
Broadleaf weed (%)			
Antas	15.6	10.8	9.15
Coolamon	19.7	5.70	3.38
Denmark	18.0	1.50	4.52
Monti	22.9	15.0	12.9
Napier	16.4	3.98	4.50
Narrikup	20.1	6.21	6.33
Trikkala	20.4	5.51	6.74

Average	19.0	6.96	6.78
P value – HB	0.004	SEM – HB	0.8013,0.8353*
P value – CV	0.073	SEM – CV	0.9875,1.040*
P value – HB*CV	0.767	SEM – HB*CV	1.846,2.014*
Grass weed (%)			
Antas	5.59	7.41	2.57
Coolamon	3.15	9.59	3.64
Denmark	1.11	7.81	1.51
Monti	4.13	5.24	4.11
Napier	3.66	3.37	1.17
Narrikup	2.07	8.12	2.06
Trikkala	1.85	7.70	2.79
Average	3.08_b	7.04_b	2.55_b
P value – HB	0.020	SEM – HB	0.8593
P value – CV	0.842	SEM – CV	1.422
P value – HB*CV	0.944	SEM – HB*CV	2.432
Dead material (%)			
Antas	0.84	3.30	6.28
Coolamon	1.47	1.60	1.52
Denmark	0.38	2.15	1.09
Monti	5.04	2.99	2.66
Napier	2.57	1.17	1.30
Narrikup	2.76	7.94	1.51
Trikkala	4.53	0.83	1.20
P value – HB	0.732	SEM – HB	0.5523
P value – CV	0.179	SEM – CV	0.8826
P value – HB*CV	0.033	SEM – HB*CV	1.446

Note - * indicates that the data was arcsine transformed and the SEM of mean had been back-transformed.

Appendix 9 Botanical composition (%) of subterranean clover cultivars on 6 December 2018 after treatment with herbicides at establishment, at Iversen 9, Lincoln University, Canterbury, New Zealand.

Cultivar	Control	Flumetsulam	Spinnaker®	Average
Subterranean clover (%)				
Antas	65.8	73.3	85.5	74.9_{b}
Coolamon	48.4	66.5	72.2	62.4_{cd}
Denmark	72.1	73.5	77.2	74.3_{bc}
Monti	46.2	68.1	42.1	52.1_{d}
Napier	88.5	86.5	90.3	88.4_{a}
Narrikup	31.4	61.7	59.8	50.9_{d}
Trikkala	41.8	57.7	67.5	55.7_{d}
P value – HB	0.052	SEM – HB	1.843,1.826*	
P value – CV	<0.001	SEM – CV	2.633,2.572*	
P value – HB*CV	0.135	SEM – HB*CV	4.151,4.023*	

Cultivar	Control	Flumetsulam	Spinnaker®	Average
White clover (%)				
Antas	13.7	13.9	8.90	12.1_{bc}
Coolamon	24.5	17.9	18.1	20.2_{ab}
Denmark	16.3	13.6	13.3	14.4_{ab}
Monti	23.5	24.3	27.7	25.2_{a}
Napier	5.85	4.15	4.49	4.83_{c}
Narrikup	36.9	16.6	22.8	25.4_{a}
Trikkala	32.5	33.5	12.8	26.3_{a}
P value – HB	0.233	SEM – HB	1.184,1.215*	
P value – CV	0.003	SEM – CV	1.882,1.979*	
P value – HB*CV	0.521	SEM – HB*CV	2.941,3.182*	

Cultivar	Control	Flumetsulam	Spinnaker®
Broadleaf weed (%)			
Antas	17.0_{cd}	6.60_{ghi}	3.01_{jk}
Coolamon	18.2_{cd}	9.18_{fg}	4.31_{hij}
Denmark	7.99_{fgh}	8.18_{fg}	6.83_{gh}
Monti	26.1_{a}	1.20_{jk}	24.2_{ab}
Napier	2.49_{jk}	3.15_{ijk}	0.22_{k}
Narrikup	25.1_{a}	16.6_{d}	10.8_{ef}
Trikkala	21.3_{bc}	3.16_{ijk}	12.9_{e}
P value – HB 0.004		SEM – HB	0.6225,0.6533*

P value – CV	0.010	SEM – CV	1.282,1.385*
P value – HB*CV	<0.001	SEM – HB*CV	1.671,1.862*
Grass weed (%)			
Antas	0.11_{de}	0.12_{de}	0.15_{de}
Coolamon	0.67_{cde}	2.02_{ab}	0.40_{cde}
Denmark	0.23_{de}	0.38_{cde}	0.00_{e}
Monti	0.10_{de}	2.17_{a}	1.09_{bcd}
Napier	0.15_{de}	0.00_{e}	0.00_{e}
Narrikup	0.87_{cde}	0.55_{cde}	0.07_{de}
Trikkala	1.34_{abc}	0.42_{de}	0.79_{cde}
P value – HB	0.051	SEM – HB	0.1017
P value – CV	0.004	SEM – CV	0.1967
P value – HB*CV	0.044	SEM – HB*CV	0.3584
Dead material (%)			
Antas	3.42	6.09	2.45
Coolamon	8.33	4.40	4.99
Denmark	3.38	4.36	2.65
Monti	4.05	4.28	4.97
Napier	2.99	6.20	4.97
Narrikup	5.71	4.58	6.55
Trikkala	3.09	5.19	5.94
P value – HB	0.668	SEM – HB	0.4563
P value – CV	0.883	SEM – CV	1.392
P value – HB*CV	0.572	SEM – HB*CV	1.884

Note - * indicates that the data was arcsine transformed and the SEM of mean had been back-transformed.